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AUX SECTIONS 11 et 12

11 Physiology
a nutrition
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SECTION 11

PHYSIOLOGIE VEGETALE

I. Physiologie de la nutrition

1. *PHOTOSYNTHESE*

PHOTOSYNTHETIC PHOSPHORYLATION, THE CONVERSION OF LIGHT INTO PHOSPHATE BOND ENERGY BY CHLOROPLASTS

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Chloroplasts removed from living cells retain for an appreciable period of time that part of the photosynthetic apparatus which is responsible for the evolution of oxygen under the influence of light (Hill reaction) in accordance with equation 1.

$$1.\quad A + H_2O \xrightarrow[\text{chloroplasts}]{\text{light}} H_2A + 1/2\ O_2$$

in which A represents an electron or hydrogen acceptor other than carbon dioxide. Evidence has now been obtained that isolated whole chloroplasts in the absence of CO_2 are also able to convert light energy into chemical

$$2.\quad H_2O \xrightarrow[\text{chloroplasts}]{\text{light}} 2e + 2H^+ + \{O\}$$

$$3.^{*}\quad 2e + 2H^+ + \{O\} + n \cdot AMP + 2n\ Pi \longrightarrow n \cdot ATP + H_2O$$

Sum :

$$4.\quad 2n\ Pi + n \cdot AMP \xrightarrow[\text{chloroplasts}]{\text{ligth}} n \cdot ATP$$

energy in the form of the high energy pyrophosphate bonds of adenosine triphosphate (ATP). The reactions are schematically represented in 2, 3, and 4. AMP and Pi represent adenosine — 5 — phosphate and orthophosphate respectively.

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* This formulation is not intended to exclude the possibility that ATP is formed by the addition of pyrophosphate to AMP.

This photochemical esterification of inorganic phosphate by chloroplasts, independent of CO_2 fixation, thus provides a mechanism for converting light energy into ATP, independent of energy released by reoxidation of partly or wholly reduced products of photosynthesis. As shown in equation 4 this photosynthetic phosphorylation is not accompanied by the evolution of oxygen. The brackets around the oxygen atom in reactions 2 and 3 are intended to denote that it has not been determined as yet whether : *a*) oxygen is released in equation 2 and immediately consumed in reaction 3, thus giving no manometrically measurable pressure change, or *b*) whether the oxygen in reaction 2 remains attached to some substance within the chloroplast, and is reduced by the electrons in accordance with reaction 3.

Photosynthetic phosphorylation (reaction 4) differs from the Hill reaction (reaction 1), in that there is no net O_2 evolution ; moreover, the reactants, phosphate and adenylate, and the product, ATP, are known cellular constituents. Photosynthetic phosphorylation also differs from the model system of oxidative phosphorylation with illuminated chloroplasts of VISHNIAC and OCHOA**. In their system illuminated chloroplasts served a limited function of reducing added diphosphopyridine nucleotide (DPN). The reduced DPN was then reoxidized by mitochondria from non-photosynthetic tissue, rat liver or mung beans. (We consider it doubtful that mitochondria from non-green portions of plants have greater relevance to photosynthetic events than mitochondria from animal tissues.) In the absence of mitochondria their system was inactive ; the omission of DPN halved the rate of the reaction. By contrast, no particles other than washed chloroplasts are involved in photosynthetic phosphorylation, nor is there any effect observed from adding DPN or TPN (triphosphopyridine nucleotide). In oxidative phosphorylation oxygen is consumed, while in photosynthetic phosphorylation there is, so far, no evidence of oxygen consumption, nor would it be expected from equations 2, 3, and 4. However, aerobic conditions were found to be necessary for continued operation of photosynthetic phosphorylation. The oxygen dependence of this reaction is being further investigated. Under favorable conditions we have obtained esterification of approximately three micromoles of inorganic phosphate per mg. chlorophyll per hour.

Photosynthetic phosphorylation has been demonstrated in a system consisting of washed spinach chloroplasts, $MgSO^4$, AMP, KCl, ascorbic acid, radioactive phosphate, glucose, and hexokinase. Heating the chloroplasts at 55°C. for 5 minutes destroyed all activity. The rate of ATP synthesis was measured by the incorporation of P_{32} into organic phosphate. In addition radioactive glucose — 6 — phosphate was identified as a product of the reaction by paper chromatography.

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A COMPARISON OF VARIOUS PHENOMENA CONNECTED WITH PHOTOSYNTHESIS (FLUORESCENCE, REDOX POTENTIALS, PHOSPHATE EXCHANGES, GAS EXCHANGE AND OTHERS) WITH SPECIAL REFERENCE TO INDUCTION EFFECTS IN CHLORELLA

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The aim of the present paper is to discuss some of the available evidence for interrelating various phenomena accompanying the process of photosynthesis in photo-autotrophic cells, as outlined in the title of the paper. Observations along some of these lines were made long ago by the senior author (1, 2, 3, 4) and have recently been corroborated and extended in our laboratory (5). Besides this, observations made elsewhere fit in very well in the general picture. Since the greater part of the observations have been made with strains of the green alga *Chlorella,* we will draw special attention to this material. The conclusions are based both on studies in the stationary state of illumination and during the so-called induction period. The general remark may be made that in our opinion the transitory changes observed in various phenomena (as e. g. those mentioned in the title of this paper) reflect changes of concentrations in essential compounds of transitory reactions (1). Thus, they show how various parts of the photosynthetic chain strive from a stationary state in darkness to another stationary state in continuous light. Parallelisation of various types of phenomena during this phase may throw light on properties (both kinetic and chemical) of partial reactions. Correlations under stationary conditions show which factors influence components of the overall process in this stage. Examples of this will be given below.

It is well known that evidence has been since long in favour of the concept that photosynthesis is built up of a light reaction and at least one (but very probably more) dark reactions. This concept has been made more definite during later years in such a way that in photosynthesis light energy was considered to be absorbed by the pigment system of the photosynthetic apparatus, yielding an excited state of this system, especially of its chlorophyllous compounds. The excitation energy was considered to be transferred to an energy acceptor, which, upon acceptation of this energy was the first compound to enter into the chain of chemical conversions ultimately leading to the reduction of carbon dioxide. This concept resulted especially from correlated studies of photosynthesis and chlorophyll fluorescence (1). Studies of this type, made with purple sulphur bacteria, were especially in favour of the conclusion that carbon dioxide in photosynthesis is, in principle, not closely connected with the act of energy transfer, whereas the relation between energy transfer and the hydrogen donor is much more direct (2). In *Chlorella,* the observation was made that more reduced states of the cell in general yield higher fluorescence values (1), and that, in normal photosynthesis, fluorescence gradually decreases in approaching the stationary state of illumination. It was also found that, at the beginning of an illumination, fluorescence invariably increased, showing the attainment of a more reduced state (« reducing action of the light ») (6, 1). Shortly after the beginning of an illumination (A) fluorescence mostly showed a definite peak (B), followed by a minimum (C), after which a renewed increase mostly followed, leading to a maximum (D), after which the final decline sets in. It is interesting to notice that in cases in which photosynthesis is somehow inhibited (e. g. in the absence of CO_2 or in the presence of cyanide) fluorescence remains virtually at the level D, whereas in normal photosynthesis the above mentioned decrease sets in after D. This is another indication that this final decline is connected with the established oxygen production in photosynthesis.

Studies with diatoms have shown that after a prolonged dark period even in air a dark reaction in the photosynthetic apparatus requires reactivation which, under the conditions of our experiments, took somewhat more than 10 sec. Similar (so far unpublished) observations were made with *Chlorella,* yielding similar time order. It was concluded (7) that this reactivation means a shift towards a more oxidized state. For the part ABC of the fluorescence time-curve, as described above, it was accepted that it was connected with a sudden evolution of oxygen as observed earlier by Beyerinck, and more recently by R. Hill. The point B then would represent the removal of an oxidized compound from the energy transfer system by the « reducing action » of the light. Considering the observations on the time course of oxygen evolution in *Chlorella* suspensions made by Frank *et al.* (8) it would seem most logical to parallelize the transitory peak in oxygen evolution with C in the fluorescence curve.

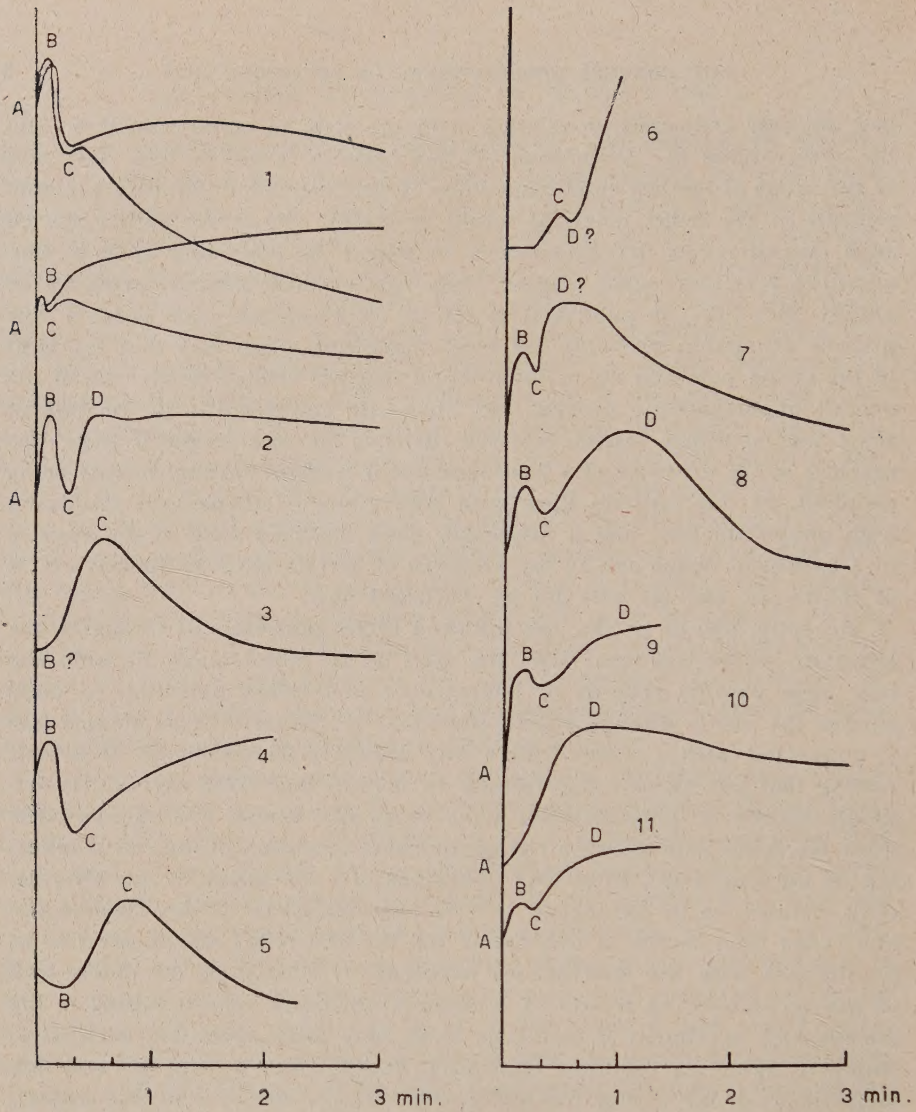
In order to be able to parallelize various induction phenomena it is necessary that they are put on the same time basis. Therefore, the times, as found more or less characteristic in our experiments, should first be mentioned. They are for *Chlorella* fluorescence curves : B : 5-10 sec. after the start of illumination, C : 10-20 sec., D : 20-30 sec. after idem.

Before proceding to a comparison of various initial effects as collected recently in our laboratory and elsewhere, we first will devote some general remarks to studies of redox potentials in suspensions of photosynthesizing micro-organisms.

It was mentioned above that the observations on *Chlorella* fluorescence had strongly indicated its relation with the state of oxido-reduction. Also the observations in *Chromatium* (2) had been in favour of this view, and in the scope of the last mentioned study it was concluded that direct measurements of the redox potential would be useful. The senior author started such measurements in *Chromatium*, in which the stationary state of illumination was thoroughly studied. Only part of these measurements (made chiefly 1945-1947) is published so far (3, 4). From the view point of our present discussion probably the most important result was that the shift of the redox potential upon illumination was not related to the rate of the overall photosynthetic process, but obviously connected with the fate of some special redox system involved. Briefly, the most reduced stage was reached in the presence of a hydrogen donor without carbon dioxide being supplied, an intermediate stage with both types of compounds, yielding a high photoreduction, and a noticeably more oxidized state in the absence of a hydrogen donor and in the presence of carbon dioxide. Special effects of inhibitors, etc. (4) will not be discussed now.

Recently, one of us (S), has followed redox potentials of *Chlorella* suspensions (under nitrogen) after the start of an illumination (5), and also has made parallel records of fluorescence and redox potential. In these studies the above discussed derivation that in *Chlorella* high fluorescence is connected with a reduced state was definitely demonstrated. It is interesting that our recent observations on fluorescence time curves are strikingly similar to those obtained many years ago. Spruit was able to determine the delay with which a change in redox condition of the cell is reflected by the electrode ; under our conditions this was about 19 sec. This finding enabled us to parallelize special characteristics of the fluorescence and redox time curves. It was found that the first redox maximum is to be parallelized with the fluorescence maximum C (shown in the slides, and in the accompanying figure). A minimum, obviously corresponding to the fluorescence maximum B manifests itself only after anaerobic dark incubation of moderate duration. Obviously, fluorescence, tracing the situation immediately at the energy transfer system, is more sensitive in this respect. But, in general, we may say that the fluorescence course ABC is accompanied by contrary shifts in redox potential. This correlation indicates that in the first 20 sec. after the start of an illumination the potential changes are chiefly determined by reactions immediately connected with the energy transfer. Lateron, after D, this strict correlation is no longer valid, indicating that parts of the reaction chain, not immediately concerned with the energy transfer process, may also act as potential determining agents.

We will now pass on to curves published by Strehler and Arnold on the « bioluminescence » of *Chlorella*, accompanying photosynthesis (9). Strehler and Arnold also studied the time course of this effect, i. e. the strength of the bioluminescence phenomenon observed immediately after various times of illumination. This time course, also in the duration of the



Comparison of various induction phenomena in *Chlorella photosynthesis* : Ordinate : arbitrary units, Abscissa : time of illumination in min.

1. fluorescence ; upper pair : in pure nitrogen, upper one with, lower one without cyanide. Lower pair : in air, upper one with, lower one without cyanide (after Wassink and Katz, ref. 1).
2. fluorescence ; according to measurements of Spruit in this laboratory.
3. redox potential(higher values = more oxidized) ; as curve 2.
4. fluorescence after a relatively short period of anaerobic incubation ; as curve 2.
5. redox potential after a relatively short period of anaerobic incubation ; as curve 2.
6. oxygen evolution, after Franck *et al.* ref. 8. Delay of apparatus about 20 sec.
7. bioluminescence of *Chlorella*, after Strehler and Arnold, ref. 9.
8. same as 7, from ref. 12.
9. bound phosphate in nitrogen atmosphere, according to unpublished measurements of J. Rombach in this laboratory (ref .10).
10. ATP-formation, after Strehler, ref. 12.
11. bound phosphate, in an atmosphere of air, unpublished experiments of J. Rombach in this laboratory (ref. 10).

essential phases (see the slides and the graph) is strikingly similar to the fluorescence curves obtained by us. As is known, the mentioned authors explain the bioluminescence as a fluorescence, due to back reactions from the links of the photosynthetic chain back to the excited chlorophyll molecule. The obvious resemblance between the time course of fluorescence and of bioluminescence seems to indicate that, during the first 20 sec. of illumination, these back reactions primarily arise from steps immediately concerned with the energy transfer reaction. This is in accordance with the view that during this time the photosynthetic chain does not yet operate in full, a conclusion which was reached earlier by observing that after 10 sec. light a dark reaction still shows only partially restored capacity (see above) and moreover by the fact that CO_2 hardly influences the part ABC of the potential and fluorescence time curves (Spruit, partly unpublished) which also can be said from cyanide with respect to the fluorescence time curve (1).

Recently, J. Rombach (10), in our laboratory, made studies on phosphate conversions after the start of an illumination. His technique was much the same as that of Kandler (11) except that samples were taken at still shorter intervals, and that his observations were made with the same strain of *Chlorella,* and under similar experimental conditions as the work of Spruit. The curves found, generally resembled those of Kandler, except that the initial effects showed up much more quickly. In nitrogen, the primary conversions are more pronounced than in air. The time course again shows a striking correlation with the fluorescence behaviour in that B of the fluorescence curve corresponds with a maximum formation of TCA stable phosphate, C with a minimum, D again, with a maximum. This indicates that during the first moments of an illumination (within 10 sec.) a reducing effect arises at the energy transfer system and that, with exactly the same time course, organic phosphate is built up. It is tempting to suggest that this phosphate represents a specific compound which is built at the energy transfer system in close connection with the act of energy transfer, and that it is closely related to the primary energy acceptor in photosynthesis.

Finally, it is now very interesting to compare Rombach's curves with the time curve of ATP-formation according to Strehler (12). It is then to be remarked beforehand that the time course of fluorescence in Strehler's *Chorella* material very probably would be like ours, since the bioluminescence curve is similar (see above). It now appears that the maximum in B in Rombach's curves is lacking in ATP formation (12, fig. 1). This leads to the conclusion that the phosphate compound to which we above described the maximum B in Rombach's curves is not ATP, but that it is superimposed on the curve of ATP formation which will partly fill up the minimum C, and be chiefly responsible for the maximum D. Investigations into the nature of the phosphate compound to which we provisionally ascribe the maximum B are now being undertaken in our laboratory.

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SOME OBSERVATIONS ON THE RELATION BETWEEN PHOSPHATE METABOLISM AND PHOTOSYNTHESIS IN *CHLORELLA*

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It has been established that light stimulates uptake of orthophosphate in *Chlorella.* Especially when CO_2 is absent we found this uptake pronounced and lasting for several hours. The greater part of the newly formed cellular phosphates can be extracted, after treatment with cold TCA, either with not diluted HCl, or (after removal of lipids with alcohol-ether) with dilute alkaline (pH 9-10). The latter treatment causes negligible hydrolysis, the extract consists for the greater part of labile phosphates. It was concluded to be poly- or metaphosphate by :

1) lability in 1n HCl, 7 min. at 100°C,
2) metachromatic reaction with toluidine blue,
3) precipitation with barium salts at pH 2-3.

Metaphosphate has already been demonstrated to be a normal constituent of *Chlorella* (HOLZER).

Several investigations have been made in order to establish the relation between light and phosphate metabolism, and to demonstrate a participation of energy-rich phosphates in CO_2-reduction (see BROWN and FRENKEL). ATP is formed immediately after the start of illumination (STREHLER). It might be conceived that the (energy-rich) polyphosphates are derived from this compound and stored as energy reserve when the energy consuming CO_2-reduction is blocked by low CO_2-tension. Metaphosphate decreases (and orthophosphate increases) in darkness when O_2 is absent. An accumulation of metaphosphate in light, and its consumption in darkness was described by STICH in the siphonocladalous alga *Acetabularia*.

Various factors (light intensities and inhibitors) known to influence photosynthesis were studied in their relation to metaphosphate formation. A rather similar sensitivity of the two processes was found with respect to DNP, NaN_3 and KCN. Differences in sensitivity were found with respect to light intensity and phenylurethane. Some conclusions will be presented.

H. HOLZER : *Z. Naturforschg.*, VI b, 424-430, 1951. — A. H. BROWN and A. W. FRENKEL : *Ann. Rev. Plant Physiol.*, IV, 46-51, 1953. — B. L. STREHLER : *Adv. Biochem.*, XLIII, 67-79, 1953. — H. STICH : *Z. Naturforschg.*, VIII b, 36-44, 1953.

KINETIC STUDIES OF PHOTOSYNTHESIS, USING A RECORDING VOLUMETER OF EXTREME SENSITIVITY

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The maximum photosynthetic rate of green cells, even if abundantly supplied with CO_2 and nutrients, is reached at much lower light intensities than that of natural sunlight. This appears to be one of the main limitations of the growth yields in algae mass-cultures (and probably of field crops as well). The effects caused by light of higher intensities therefore were further analysed.

A modified volumetric method, combining features of extreme sensitivity with easy operation and automatic recording of gasexchanges, was developed and used for these studies. Pressure changes in a reaction vessel are automatically compensated by a variation of the volume of a compensating vessel. The latter variation is brought about by a recorder motor, coupled with the recorder pen. Motor movement is governed by the index meniscus via a photoelectric detector. With larger (0.2-1 ml) samples high relative sensitivity can be attained ; on the other hand, micro reaction vessels of very simple construction have been successfully used, obtaining sensitivities of about 0.01 μl corresponding to a full scale span of the recorder.

The relation between light intensity and assimilation rate is practically linear uptil or over the point at which half the saturation rate is reached. At still higher intensities the curve bends over rather sharply and reaches a level, which is constant over quite an extended intensity range.

This type of curve cannot be explained by accepting a single first order enzymatic reaction as the rate limiting step in photosynthesis. On the other hand, however, such a step was found in flashing light experiments. The limiting mechanism, which decreases light energy utilisation, thus appears to have a complex character. Phenomena, correlated with photo-oxidation, could possibly play a role here. It appeared, that upon exposure to very intense light, photosynthesis starts at a rate equal to the saturation value ; this rate, however, decays with time. At intermediate intensities a final positive rate of O_2 exchange obtains, but at higher intensities O_2 consumption (as in darkness) finally results. This decay of the photosynthetic rate appeared to have first order character, the time constant being inversely proportional to the intensity applied. At the same time it could be shown that only light beyond a critical intensity induces photo-oxidation effects of this character. Since this latter intensity is several-fold higher than the saturating intensity, it was concluded, that photo-oxidation is not directly involved in limiting the photosynthetic rate.

Further analysis of the saturation phenomena has been made with the aid of flashing light experiments.

THE MECHANISM OF PENTOSE PHOSPHATE CONVERSION TO HEXOSE MONOPHOSPHATE WITH A PEA LEAF AND PEA ROOT PREPARATION*

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The conversion of ribose-5-phosphate-(1-C^{14} or -2, 3-C^{14}) to hexosemonophosphate by pea root and pea leaf preparations has been investigated.

Preparations of roots converted pentose phosphate-1-C^{14} into 1, 3-labeled hexosemonophosphate, with 70 per cent of the isotope in the 1-position.

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* Research carried out at the Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

Pentose phosphate-2, 3-C^{14} was converted by the root extracts into hexosemonophosphate with radioactivity in 4 of the 6 carbon atoms. Nearly one-half of the total activity appeared in carbon 4. Carbon atoms 2 and 3 were approximately equally labeled, and a small but significant amount of activity was found in carbon 1.

The leaf preparations converted pentose phosphate-1-C^{14} into hexosemonophosphate labeled predominantly in carbon 1 (63 per cent) and carbon 3 (19 per cent). However, in contrast to the preparation of root extracts, both carbon 4 as well as 6 contained 9 per cent of the total radioactivity. Hexosemonophosphate formed from pentose phosphate-2, 3-C^{14} was labeled in the following manner with the numbers representing the per cent of the total radioactivity : carbon 1 (3), carbon 2 (13), carbon 3 (36), carbon 4 (43), carbon 5 (5), while carbon 6 was unlabeled.

The similarity in distribution of label in the glucose-6-phosphate formed from pentose phosphate-(1- and -2, 3-C^{14}) by crude extracts of rat liver (Horecker B. L., Gibbs M., Klenow H. and Smyrniotis P. Z., *J. Biol. Chem., in press*) and by extracts of pea roots is taken as evidence for a similar sequence of reactions. In addition to the transketolase reaction in which an « active glycol-aldehyde » group is transferred from ribulose-5-phosphate to ribose-5-phosphate to form sedoheptulose-7-phosphate and the transaldolase reaction whereby an « active dihydroxyacetone » group is transferred from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate, the chlorophyll containing tissues catalyze an additional reaction which leads to the appearance of carbon atom 1 of pentose phosphate in carbon atoms 4 and 6 of the hexose phosphate. This mechanism will be discussed.

This work yields a first indication as to mechanisms whereby the isotope initially incorporated into the 3, 4 position of the hexosemonophosphate during photosynthesis in $C^{14}O_2$ may spread to the other positions of the hexose.

PHOTOSYNTHESE ET METABOLISME DES ACIDES ORGANIQUES CHEZ LE *BRYOPHYLLUM*

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On sait, d'après les travaux de M. Thomas et de ses collaborateurs, que les feuilles des Crassulacées peuvent fixer l'anhydride carbonique, à la lumière comme à l'obscurité, par une réaction de Wood-Werkman, indépendante de la photosynthèse.

En présence de lumière, ces feuilles peuvent réaliser simultanément :

a) des oxydations cellulaires du type respiratoire ;

b) une fixation de CO_2 par β-carboxylation, avec accumulation d'acide malique principalement, par un mécanisme comparable à celui qui est catalysé par l'enzyme malique d'Ochoa ;

c) une oxydation des acides accumulés ;

d) la photosynthèse.

Nous avons suivi, chez les feuilles de *Bryophyllum Daigremontianum* Berger, les variations quantitatives des équilibres réalisés entre ces différents phénomènes, au point de vue des échanges de gaz, de l'acidité et de la teneur en acides organiques divers (analyse chromatographique des acides malique, citrique, isocitrique, fumarique, succinique). Les feuilles étaient mises en présence d'atmosphères riches en CO_2 ou dépourvues de ce gaz, et présentant, de plus, différentes tensions partielles d'oxygène, allant de 0 à 100 % (température constante, T = 19°C, éclairage constant : 2000 lux).

1) En l'absence de CO_2 externe, la photosynthèse s'effectue à partir du CO_2 produit par une oxydation au moins partielle des acides organiques, notamment de l'acide malique.

2) En présence de CO_2 externe (5 à 6 %), la photosynthèse suit un cours indépendant de l'oxydation des acides organiques et le quotient photosynthétique mesuré est égal à l'unité, quelle que soit la tension d'oxygène dans l'atmosphère.

3) En anaérobiose et en l'absence de CO_2 externe, la photosynthèse est ralentie à la fois en raison de l'affaiblissement propre de l'appareil photosynthétique et en raison de la carence en CO_2 résultant de la lenteur et de l'oxydation des acides organiques.

4) En atmosphère fortement oxygénée et en l'absence de CO_2 externe, le CO_2 émis par les oxydations des acides n'est que partiellement repris par la photosynthèse. Le point de compensation lumineux oxydation des acides / photosynthèse est plus élevé que le point de compensation lumineux respiration / photosynthèse.

L'analyse des acides organiques a permis de préciser les modalités de la concurrence entre la photosynthèse et la carboxylation vis-à-vis de CO_2 et les modalités des oxydations nécessaires à la reprise active du carbone des acides dans le processus photosynthétique.

EFFETS DE LA THIOUREE SUR LA REDUCTION DES NITRATES ET LA PHOTOSYNTHESE D'UNE ALGUE VERTE

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et Alexandre **Bagdiantz** (*Téhéran*)

La thiourée possède une propriété cytostatique à l'égard de nombreuses Chlorophycées. Jusqu'à concurrence de 1 mgr. par litre de solution nutritive, la molécule n'exerce pas d'effet visible sur le développement de l'inoculum. Des doses allant de 1 mgr. à 10 mgr. provoquent, dans le milieu de culture liquide non aéré, des retards de la multiplication proportionnés à la concentration du poison. 100 mgr. par litre assurent une cytostasie définitive.

Pour comprendre le mécanisme de cette inhibition de la croissance, nous avons tenté de la prévenir en complétant, avant l'inoculation, la solution nutritive intoxiquée par des systèmes protecteurs. Dans le cas des Algues, nous en avons découvert trois, qui ne semblent, au premier abord, n'avoir point de relations entre eux : surcharge en métaux oligodynamiques, complément de la solution nitratée par une source supplémentaire d'azote non nitrique, incorporation d'un donateur d'hydrogène dans la composition minérale du milieu de culture.

L'annulation presque totale, dans nos expériences, de l'inhibition par addition de métaux tels que le Mn, le Zn, le Cu, indique que la disponibilité du nitrate peut donc être rétablie. L'expérience tend à prouver que cette disponibilité dépend de la liberté de divers effecteurs métalliques qu'en complexant la thiourée soustrait aux systèmes enzymatiques qui en ont besoin (nitrate-réductase).

Lorsqu'on complète la solution nitrique intoxiquée, où le nitrate de calcium fait figure d'unique source d'azote, par du nitrite de potassium ou du chlorure d'ammonium ou des acides aminés, on permet un développement quasi normal de l'Algue en présence de la dose antigénétique de thiourée. Tout se passe comme si l'intoxication affectait essentiellement la réduction des nitrates en nitrites. Le mécanisme d'intervention de la thiourée devant rester le même, la probabilité devient grande pour que cette molécule paralyse les enzymes de réduction par suppression des métaux auxiliaires.

On peut encore prévenir, dans une large mesure, l'intoxication des Algues par la thiourée en ajoutant à la solution minérale de Detmer 1/3 du glucose ou du pyruvate ou du lactate de sodium.

L'interprétation de ces faits exige le rappel des propositions suivantes :

toute réduction d'un nitrate réclame le concours d'une molécule donatrice d'hydrogène. C'est le sucre issu de la photosynthèse qui joue ce rôle dans la nutrition des Algues. Tout se passe, en présence de thiourée, comme si le milieu manquait d'un donateur d'hydrogène, puisque l'apport d'une molécule organique oxydable rétablit la croissance. Cette carence en milieu intoxiqué vient de ce que le sucre n'a pu se former ou que, s'étant formé, son utilisation est devenue impossible. Nous verrons plus loin que la thiourée réduit la photosynthèse.

Les trois artifices sus-mentionnés permettent, à des degrés divers, de protéger l'Algue contre l'intoxication par la thiourée. Le tableau ci-joint propose une opinion sur les relations existant entre ces divers phénomènes :

Conditions :	Thiourée	Protecteurs :	Système enzymatique :	Résultat (NO_2 formé)
normale	—	—	R-NO_3 + H_2- DONATEUR Me-enzyme	+++
intoxiquée	+	—	R-NO_3 + ... enzyme	—
protégée	+	**R-NO_2**	R-NO_3 + ... enzyme	+++
protégée	+	**Me**	R-NO_3 + ... **Me**-enzyme	+
protégée	+	**H_2- DONATEUR**	R-NO_3 + **H_2DONATEUR** .... enzyme	++

Me-enzyme = nitrate-réductase (holoferment).
En lettres grasses : compléments exogènes.

Dans la condition intoxiquée, le schéma qui précède tient compte, au minimum, d'une double perte qui affecte la nitrate-réductase : complexion du métal-coferment et carence d'un donateur d'hydrogène. La protection par l'addition de nitrite ou d'une source encore moins oxydée d'azote, consiste à se passer de l'appareil enzymatique altéré et à fournir directement à l'Algue la molécule que la nitrate-réductase aurait contribué à préparer dans les conditions normales.

La protection par addition de métaux est toujours partielle. Le rétablissement de cette fonction catalytique laisse, par conséquent, l'enzyme encore affaibli. Ce fait plaide encore en faveur de la double altération postulée plus haut. Nous tenons d'ailleurs pour assez grossière l'image simple d'un métal soustrait directement à l'holoferment. Il est encore impossible d'affirmer si la carence métallique affecte d'emblée le co-ferment ou si elle intervient dans des réactions synergiques accompagnant la réduction proprement dite du nitrate. L'absence d'une spécificité métallique étroite dans ces expériences est favorable à cette réserve.

La protection par addition d'un donateur d'hydrogène est plus efficace. A supposer que, dans cette circonstance, le métal reste inactif par complexion, l'Algue paraît en souffrir peu. Certes, elle ne peut se dispenser de prendre l'azote nécessaire à cette unique source nitrique. On peut alors se demander si les deshydrogénases mises en jeu par la présence d'un donateur d'hydrogène ne remplacent pas, jusqu'à un certain point, la nitrate-réductase altérée. Ces deshydrogénases de la respiration sont d'ailleurs reconnues comme indifférentes aux carences métalliques.

Vue sous l'angle plus général de la biochimie, la question étudiée ici appelle les remarques suivantes : toute cellule possède normalement des thiols solubles, des thiols fixés (protides), des métaux-traces et des corps quinoniques. C'est à l'égard de ces trois types de constituants cellulaires que s'exerce, jusqu'à plus ample informé, l'intoxication par la thiourée. La pénétration en quantité excessive dans la cellule de thiols solubles, venus du milieu, fait concurrence à l'oxydation naturelle des thiols endocellulaires. Premier dommage. Cette oxydation s'opère d'ailleurs avec le concours de métaux lourds qui sont eux-mêmes bloqués par la pléthore des molécules thiols. L'accaparement de cette réserve métallique endocellulaire par la thiourée a des conséquences enzymatiques multiples. Deuxième dommage. Enfin, la réduction massive des quinones endocellulaires par les thiols exogènes crée de son côté d'importantes pertubations. Troisième dommage.

L'hypothèse d'un mécanisme unique d'intervention de la thiourée doit être écartée. De cette pluralité d'effets — plus ou moins liés entre eux — découle le principe de la pluralité d'agents protecteurs, principe dont nous avons fourni la démonstration au cours de ce travail.

La place manque ici pour relater les résultats obtenus dans l'étude de l'intoxication de la fonction photosynthétique par la thiourée. Cette inhibition, que nous n'avons pu jusqu'à présent prévenir par l'apport de molécules donatrices d'hydrogène et par la surcharge métallique du milieu, s'inscrit dans le jeu des carences que nous avons décrites pour la réduction des nitrates. Nos expériences montrent enfin que la thiourée intervient au titre d'effecteur négatif dans la photolyse chlorophyllienne.

CHANGE OF PHOTOSYNTHETIC ACTIVITY OF *CHLORELLA* CELLS DURING THEIR NORMAL LIFE CYCLE

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It has been reported (*Bioch. et Bioph. Acta, 12,* 1953, 23) that the cells of *Chlorella* assume, in the course of their growth, two distinct forms possessing widely different characteristics. One form, which was called « dark cells », is smaller in size, richer in chlorophyll content, and stronger in photosynthetic activity than the other, which was referred to as « light cells ». If provided with light and adequate nutrient substances, dark cells increase in mass and grow into light cells which in turn divide into a number of dark cells. This latter process involves no increase of cell mass and occurs only under aerobic conditions, irrespective of whether the cells are in the light or in the dark. It was shown that the over-all growth process of the algae is accomplished by the repetition of the mutual change between these two kinds of cells.

Comparison was made of these cells in respect to various aspects of their photosynthetic activity. While the dark cells show a photosynthetic quotient (O_2/-CO_2) of around unity (0.83-1.15), light cells give more or less larger values of the quotient. At a certain stage in the development of light cells, in which probably the most significant formative change of cell organization in forming autospores takes place, the photosynthetic quotient increases enormously, attaining values as much as 2.0-4.0. At this stage, the light-saturated photosynthetic rate shown by light cells is only 1/3-1/6 and 1/10-1/15 in terms of O_2-output and CO_2-uptake, respectively, compared with that displayed by dark cells. The striking decrease of CO_2-uptake by light cells can also be demonstrated by an experiment using C_{14}-labelled carbon dioxide. The activity of dark CO_2-fixation after the pre-illumination of algal cells (in the sense of Calvin and Benson) was also found to diminish considerably at the corresponding stage of light cells.

The light-limited rate of photosynthesis as well as the chlorophyll content of cells also decreases more or less when the dark cells transform into light cells. If the light-limited photosynthetic rate per unit mass of algal cells and per unit intensity of light (measured in terms of lux) is expressed by αo_2 or αco_2 according as it is referred to the O_2-output or $C0_2$-uptake, and if the quantity of chlorophyll contained in a unit mass of algal cells is denoted by C, then the ratio $\alpha o_2/C$ or $\alpha co_2/C$ may be regarded as representing the *relative quantum efficiency* of photosynthesis, in so far as chlorophyll represents the principal factor in causing the light absorption by algal cells. It was found that, on the average, the values of α_{O_2}/C and α_{CO_2}/C for light cells were about 1/2 and 1/3, respectively, of the corres-

α_{CO_2}/C for light cells were about 1/2 and 1/3, respectively, of the corrres-

ponding values shown by dark cells, a fact which must be duely taken into account when studying the quantum efficiency of photosynthesis in algae.

It should be added that in respect to respiration in the dark, light cells show twice as much activity (referred to a unit weight of cells) as the dark cells. The respiratory quotient ($CO_2/-O_2$) remains, however, the same, being almost equal to unity, in both kinds of cells.

ON THE COMPOSITION OF THE CELL MATERIAL AND THE LIGHT ENERGY CONVERSION DURING GROWTH OF *CHLORELLA* IN RELATION TO NITRATE SUPPLY

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The oxygen production by growing cultures of *Chlorella* decreases sharply after the nitrate in the medium is exhausted. The efficiency of light energy conversion during growth is decreased by nitrate deficiency and especially so at higher light intensities. At the same time the nitrogen content of the cells decreases also (e. g. from 10 to 3 per cent). The composition of the cell material shifts from a high protein to a high carbohydrate content, while the fat content does not change.

When cells are exposed to various light intensities in a nitrate free medium, the oxygen production of cultures at light saturating intensities drops to very low values after some time and the nitrogen content of all cultures is reduced to 2-3 per cent. This sharp decrease in efficiency with increasing light intensities is especially marked when the nitrogen content of the cells is low at the start of the experiment. In experiments of short duration the carbohydrate content then strongly increases ; in longer periods the fat content can increase up to 35 per cent. Higher fat contents have not been obtained even not by transferring old cultures into a nitrogen free medium.

Fat contents up to 55 per cent can be reached by cultures growing during long periods in a medium normally supplied with nitrate at the start. The composition of the cell material first shifts from a high protein to a high carbohydrate content, while later on carbohydrate is converted into fat. This involves a reduced efficiency of light energy conversion.

L'ANALYSE DE LA CROISSANCE EMPLOYEE DANS L'ETUDE DE LA PHOTOSYNTHESE DES PLANTES LIGNEUSES

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L'étude écologique de la photosynthèse et de la croissance chez les plantes supérieures peut être basée sur l'accroissement en matière sèche de plantes entières par unité de temps.

Les méthodes de l'analyse de la croissance (« growth analysis ») ont été conçues et développées afin d'étudier la croissance et le rendement des plantes au moyen des fonctions de la croissance qui sont : la vitesse d'assimilation nette (— net assimilation rate, NAR —, exprimée en grammes de matière sèche qui s'accumulent dans la plante par unité foliaire et par jour) ; la vitesse de croissance relative (— relative growth rate, RGR —, exprimée en grammes de matière sèche qui s'accumulent dans la plante par unité de plante et par jour) ; et le rapport de surface foliaire (— leaf area ratio, L/W — le rapport entre la surface foliaire et le poids sec total de la plante). Les méthodes d'analyse de la croissance et leurs usages ont été passés en revue récemment par Watson (8). Ces méthodes ont été employées surtout dans l'étude des grandes cultures ou des cultures sarclées, et rarement dans l'étude des plantes ligneuses comme le Cacaoyer et le *Citrus* (3, 4, 6, 7).

Comme les échantillons de plantes ligneuses ne peuvent être grands, l'effet des différences de dimensions parmi les individus est considérable. Or, il faut comparer une variable (par ex. le poids de la plante entière) mesurée sur deux groupes d'individus, le premier déraciné au commencement, le deuxième au bout d'un intervalle de quelques jours ou semaines. Il est parfois nécessaire (pour égaliser les dimensions initiales des deux groupes) de mesurer sur les *deux,* au *début* de l'intervalle, un attribut de grandeur étroitement lié au poids sec (par ex. la surface foliaire). Les poids secs des deux groupes peuvent être ainsi amenés aux niveaux correspondant à la moyenne générale de cet attribut mesuré au commencement de l'intervalle. Ce procédé, qui implique considérablement plus de travail (le calcul des équations de régression), a été employé par Goodall (2, 4) et par Monselise (6, 7) et a été examiné et discuté par McIntyre et Williams (5).

La croissance par poussées (flushes), qui est une caractéristique des plantes ligneuses, cause des complications dans l'analyse de la croissance. Il est possible que cette caractéristique soit une des causes de la marche erratique des courbes de la NAR et de la RGR du Limettier doux (6). En effet, des plantes différentes appartenant au même échantillon pourraient se trouver à des phases différentes de l'accroissement cyclique. La question de la croissance par poussées exigera beaucoup d'attention de la part des chercheurs qui emploieront l'analyse de la croissance en étudiant les plantes ligneuses.

Nous en venons maintenant à décrire quelques résultats obtenus avec ces méthodes. NAR, et spécialement RGR, sont beaucoup plus basses dans le cas des *Citrus* (7) et surtout du Cacaoyer (4) qu'avec les plantes annuelles. Une RGR très basse pourrait bien être une caractéristique des plantes ligneuses. Parmi les espèces du genre *Citrus* examinées en pépinière, nous avons trouvé chez le Bigaradier — à croissance très lente à cet âge — une NAR plus basse que chez le Limettier doux et le « rough lemon ». La RGR est aussi plus basse mais, en ce cas, la différence n'est pas significative au point de vue statistique. Des surfaces assimilatrices plus étendues (c'est-à-dire un L/W plus haut) compensent partiellement les valeurs plus basses de la NAR. A part les différences entre espèces, on a pu établir (6) l'effet d'un facteur extérieur, l'illumination, sur la NAR et la RGR du Limettier doux. Les valeurs de la RGR du Limettier doux poussant à 20 % de la lumière du jour étaient plus hautes que celles du Limettier doux poussant en pleine lumière. Quant aux valeurs de la NAR, on n'a pu constater aucune différence significative. Ces faits s'accordent partiellement avec la définition nouvelle des plantes d'ombre, donnée par Blackman et Wilson (1), puisque les agrumes sont des plantes semi-sciaphiles.

L'analyse de la croissance peut donc fournir des résultats intéressants avec les plantes ligneuses et il est souhaitable que l'on fasse des recherches plus étendues en ce domaine.

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SUR LES RAPPORTS ENTRE QUELQUES CONDITIONS DU MILIEU, LE METABOLISME DE LA CHLOROPHYLLE ET LE DEVELOPPEMENT DES VEGETAUX

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Nous avons étudié la formation de la chlorophylle, non à partir d'organes étiolés, mais dans des limbes normaux cultivés dans diverses conditions de milieu. Nous avons aussi suivi la dégradation du pigment dans la feuille, ainsi que dans des extraits complexes. On peut résumer comme suit nos résultats, recueillis principalement sur *Fragaria vesca* L., var. *semperflorens* Duch. :

I. Dans chaque feuille, la teneur en chlorophylle, d'abord extrêmement basse dans le primordium, augmente progressivement au fur et à mesure de la croissance, puis de l'étalement du limbe. La teneur est maxima dans le limbe largement étalé, et elle tend à s'abaisser lentement dans le limbe vieillissant. On trouve souvent dans le limbe jeune, à côté des chlorophylles a et b, une quantité notable de phéophytine, dont la présence est révélée par la chromatographie sur papier. Le rapport chlorophylle a/chlorophylle b varie avec l'âge. Parallèlement, l'état de liaison du pigment avec les protéines des chloroplastes change.

II. Au cours d'une seule journée, la teneur en chlorophylle totale d'un limbe donné se modifie. L'amplitude des variations de teneur peut atteindre 25 % de la valeur moyenne. Normalement, on observe une période d'accumulation nette entre 8 h. et 14 h.

III. L'accumulation de la chlorophylle est plus ou moins fortement affectée — selon les stades du développement — par la modification de la longueur des jours ou de la température ambiante, ou encore de l'humidité du sol. On peut provoquer l'élévation de la teneur jusqu'à une valeur double de la valeur normale, aussi bien que le phénomène inverse. En particulier, le maintien d'une température diurne basse — ou d'une température nocturne élevée — est nuisible à l'accumulation du pigment ; il suffit d'élever fortement la température pendant la journée, entre 10 et 14 h., pour accroître notablement l'accumulation par rapport à des témoins maintenus à une température diurne basse.

IV. L'accumulation de la chlorophylle dépend de l'état de développement de la plante. La plantule accumule relativement peu de pigment, mais l'accumulation augmente progressivement jusqu'aux environs de la floraison. Au moment de la floraison et par la suite, la capacité accumulatrice diminue. Les rapports entre les conditions du milieu et l'accumulation varient parallèlement.

V. La dégradation du pigment dans les vieilles feuilles conduit à la formation de phéophorbide et de chlorophyllide, comme le montre la chromatographie directe des limbes et des extraits totaux sur papier. Ces produits sont des intermédiaires de la dégradation ; on peut suivre leur formation *in vitro* dans un extrait acétonique complexe, contenant la matière sèche des limbes (« Blattmehl » de Willstätter). Une microméthode de mesure de l'activité de la chlorophyllase a été mise au point, et l'étude de cette activité a été entreprise en rapport avec le métabolisme chlorophyllien.

RAPPORT

RECENT INVESTIGATIONS OF PHOTOSYNTHESIS AND THE HILL REACTIONS

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Recent research on the mechanism of photosynthesis is reviewed with emphasis on the contributions arising from chloroplast studies. The physical organization of the chloroplast and of grana is discussed with reference to pigment orientation and the Emerson-Arnold « photosynthetic unit » concept. The function of phototaxis is compared in the chloroplasts of higher plants, and in unicellular algae, dinoflagellates and bacteria. Many characteristics of the normal process of photosynthesis are discussed with reference to the Hill reaction (response to mechanical injury, flashing light, temperature, mineral nutrients, enzyme inhibitors, and oxygen ; induction phenomena, the ontogenetic factor, energy transfer mechanisms and maximum photochemical efficiency or quantum yield). The partial duplication of normal photosynthesis and of photochemical nitrate assimilation *in vitro* with reaction mixtures containing isolated chloroplasts, pyridine nucleotides and cytoplasmic enzymes is reviewed and compared in detail with recent isotope studies of photosynthesis in living cells. Evidence for the participation of pyridine nucleotides and α-lipoic acid in photosynthetic hydrogen transport is reviewed. The attainment of vastly higher rates of water photolysis and oxygen evolution in the Hill reaction than in photosynthesis in comparable algal suspensions in saturating light is also reported.

SOME PROPERTIES OF CHLOROPHYLL OUTSIDE THE CELL IN RELATION TO ITS ROLE IN PHOTOSYNTHESIS

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1. Experiments will be described on the preparation of chlorophyll, bacteriochlorophyll, ethyl chlorophyllide and methyl bacteriochlorophyllide in colloidal and crystalline forms, and as monolayers. Electron microscopy, absorption spectroscopy, and x-ray diffraction are shown to be more

reliable criteria of crystallization than microscopic observation. The two- or three- dimensional, orderly forms possess spectral bands shifted by up to 100 mμ from their position in disorderly, dissolved or colloidal states. These shifts can be interpreted as due to resonance coupling between the pigment molecules in an orderly array. Comparison with the spectral properties of chlorophyll in the living cell can be made, in consideration of the hypothesis that chlorophyll is present in the chloroplasts as a monomolecular layer between a protein and a lipoid stratum in the grana. The possible role in photosynthesis of resonance energy migration between pigment molecules will be discussed.

2. The spectroscopic and photochemical properties of chlorophyll and its derivatives in solution will be discussed. Experiments on the reversible oxidation and reduction of chlorophyll and its derivatives will be summarized and related to the molecular structure of these derivatives, as elucidated, *i. e.*, by infrared absorption spectra.

CHROMATOGRAPHIE SUR PAPIER DES PIGMENTS CHLOROPHYLLIENS

par Robert Douin

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Les méthodes de chromatographie sur papier qui ont été préconisées depuis 1949 pour séparer les constituants de la chlorophylle ne permettant pas les recherches que nous nous proposions d'entreprendre, nous avons mis au point une méthode nouvelle qui repose sur un cas particulier de la chromatographie de partage.

L'extraction du pigment total est faite dans l'acétone par broyages répétés au mortier avec du sable fin. Aux solutions acétoniques réunies dans un entonnoir à décantation, on ajoute un volume double d'eau glacée et un volume égal de sulfate d'ammonium à 20 %. Les pigments sont repris dans quelques cm^3 d'éther de pétrole. Après décantation, on soutire la solution acétonique et on recueille la solution de chlorophylle dans l'éther. L'opération est répétée jusqu'à épuisement des pigments que marque la décoloration complète de la solution acétonique. Les solutions de chlorophylle brute recueillies sont ensuite séchées sur sulfate de soude. Toutes ces manipulations doivent être effectuées à basse température pour éviter les isomérisations.

Le développement est réalisé en chromatographie ascendante à une dimension sur une bande de papier en utilisant comme solvant l'alcool méthylique à 100°. L'opération est conduite dans un tube spécial dont la paroi est perforée de trous disposés en quinconce. La bande de papier humidifiée jusqu'à obtenir un taux d'hydratation de 10 % environ reçoit à 1 cm de son extrémité inférieure un volume connu de la solution éthérée du pigment total que l'on dépose transversalement sur toute la largeur de la bande. On introduit alors dans le tube à chromatographie 5 cm^3 d'alcool méthylique et on place la bande de papier verticalement au milieu du tube, son extrémité inférieure effleurant le fond, son extrémité supérieure étant fixée par un dispositif particulier. Le tout est mis dans un récipient clos en atmosphère humide. Le développement commence aussitôt et dure de 20 à 30 minutes. L'expérience montre que pour obtenir le meilleur résultat il y a lieu d'arrêter l'opération lorsque le front du solvant a atteint 12 à 15 cm de hauteur. D'une façon générale, les pigments se séparent et se placent à des hauteurs respectives correspondant à leur degré de solubilité dans l'alcool méthylique. Ils se disposent en bandes parallèles, transversales, plus ou moins larges, séparées par des intervalles blancs. Les composants de la chlorophylle sont superposés, les carotènes restant au-dessous des chlorophylles, tandis que les xanthophylles se situent au-dessus.

Dans le cas d'un chromatogramme obtenu, par exemple, à partir de feuilles de Tilleul, on observe de bas en haut cinq bandes transversales : un carotène orangé, le composant a de la chlorophylle, le composant b, une xanthophylle jaune orangé, une xanthophylle jaune. On peut, partant des organes chlorophylliens, obtenir un bon chromatogramme en deux heures. Si dans le chromatogramme sont apparues des bandes d'impuretés, qui sont généralement grisâtres, il est facile de les éliminer en les découpant, puis en reprenant les pigments dans un solvant approprié et en procédant à un deuxième chromatogramme. La méthode permet aussi la séparation des divers constituants en découpant les bandes correspondantes et en les reprenant séparément.

Divers facteurs influencent le développement : ce sont notamment la quantité de pigment total utilisée, le titre du solvant de développement, la teneur en eau du papier chromatographique.

VARIATION DU CONTENU EN CATALASES DES FEUILLES D'ARBRES FRUITIERS CHLOROTIQUES INJECTÉES DE SULFATE FERREUX

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On étudie le contenu en catalases des feuilles d'arbres fruitiers (pommier, pêcher, poirier et cognassier) normales et déficientes en fer. On déduit, selon les renseignements obtenus, que les feuilles normales contiennent plus de catalases que les feuilles déficientes en fer.

Des plantes chlorotiques, à cause de leur déficience en fer, ont été injectées avec du sulfate ferreux, selon la méthode d'injection pétiolaire du Dr Roach.

On a fait des déterminations du contenu en catalases et en chlorophylle de ces plantes injectées. L'injection du sulfate ferreux provoque une augmentation de catalases au cours des 48 heures suivant la vérification de l'injection. L'injection occasionne une augmentation de la quantité de chlorophylle des plantes déficientes, augmentation que l'on peut observer 96 heures après l'injection.

INFLUENCE DE LA LUMIERE SUR L'ACTIVITE CATALASIQUE DES SUSPENSIONS DE CHLOROPLASTES

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Cette étude a pour point de départ la recherche d'une relation entre l'activité photosynthétique et l'activité catalasique des suspensions de chloroplastes. On a souvent admis qu'il existe un rôle protecteur de la catalase vis-à-vis du mécanisme photosynthétique. En fait, je n'ai trouvé qu'un parallélisme assez grossier entre les décroissances de l'activité photosynthétique et de l'activité catalasique au cours du stockage des préparations. Par contre, une particularité intéressante est apparue : il existe une influence de la lumière sur l'activité catalasique des suspensions de chloroplastes. C'est cette propriété que j'ai par la suite étudiée et que je vais décrire ci-dessous.

Des préparations de chloroplastes d'Epinard ont été effectuées suivant la méthode classique utilisée pour la réaction de Hill : à 0°C, broyage rapide au Mixer des feuilles dans du tampon phosphate 10^{-2} M pH $\backsim$ 6, 5, élimination des débris cellulaires et lavages répétés par centrifugation à environ 10.000 g et remise en suspension dans le même tampon. Les suspensions étaient finalement conservées à 0°C. Les déterminations de l'activité catalasique ont été également faites suivant la méthode classique de Euler et Joseph-Son (1) légèrement modifiée : à partir de 25 cc de H_2O_2 10^{-2} M plus 0,55 cc de suspension de chloroplastes, les prises successives, après mélange avec un volume fixe de SO_4H_2 et centrifugation, étaient alors titrées manganimétriquement sur un aliquot du surnageant clair. Enfin, quelques mesures volumétriques ont été faites sur un appareil pourvu d'une agitation très énergique et ont confirmé les résultats de la méthode précédente. Contrairement à la catalase moléculaire (1), la catalase chloroplastique donne une cinétique tout à fait monomoléculaire pour des durées de l'ordre de 10 à 15 minutes, de sorte que j'ai souvent pu déterminer la constante de vitesse au moyen de deux points seulement. Cette simplification s'est avérée très utile pour tout travail en série sur l'influence de la variation d'un des facteurs déterminant le phénomène.

D'une manière générale, la lumière a pour effet de diminuer la constante de vitesse $k = \frac{1}{t} \log_{10} \frac{(H_2O_2)o}{(H_2O_2)t}$ de la décomposition de H_2O_2 par la catalase chloroplastique. Cette diminution est au maximum de 30 à 40 % dans les conditions où je me suis placé. Elle est affectée par différents facteurs dont voici les influences :

Longueur d'onde. — Bien qu'aucune étude systématique ne m'ait encore été possible dans ce domaine, j'ai cependant utilisé trois régions du spectre visible : rouge ∽ 660 mμ, verte ∽ 540 mμ et bleue ∽ 450 mμ isolées par des filtres sur la lumière convenablement condensée d'une lampe à arc LCT ou d'une lampe à Hg Philora. Ces trois groupes de radiations avaient des effets très comparables à intensités incidentes sensiblement égales.

Intensité. — En fonction d'intensité croissante, le phénomène semble suivre une loi de saturation typique (Fig. 1). L'intensité 100 correspond à 16.000 ergs/sec.-cm², $\lambda = 546$ mμ.

Concentration en chloroplastes. — L'activité catalasique à l'obscurité est proportionnelle à la concentration en chloroplastes, s'il s'agit d'une même préparation (en négligeant d'ailleurs l'évolution propre de celle-ci). Mais également, en première approximation, j'ai observé d'une préparation à l'autre un rapport relativement constant de k à la concentration en chlorophylle dans la solution étudiée :

$$\frac{k\ \text{sec}^{-1}}{\text{chlorophylle mg/cc}} = 5.10^{-4} \pm 1{,}5.10^{-4}$$

En fonction de la concentration en chloroplastes, l'effet de la lumière exprimée par la différence Δk entre les activités catalasiques à l'obscurité et à la lumière est sensiblement linéaire (fig. 2), autrement dit $\Delta k/k_{\text{obscurité}}$ varie fort peu. Il faut noter à ce propos que l'agitation mécanique vigoureuse utilisée dans mes expériences permet en moyenne à chaque chloroplaste de parcourir un cycle complet d'illumination, c'est-à-dire que le nombre de chloroplastes exposés à une certaine intensité pendant une durée quelconque est simplement proportionnelle à la concentration des chloroplastes. Si la modification apportée par la lumière n'est que lentement renversable à l'obscurité, c'est-à-dire dans les couches profondes, cette durée aura peu d'importance et l'effet sera lui-même proportionnel à la concentration des chloroplastes. La courbe d'absorption reportée en même temps que Δk (fig. 2)

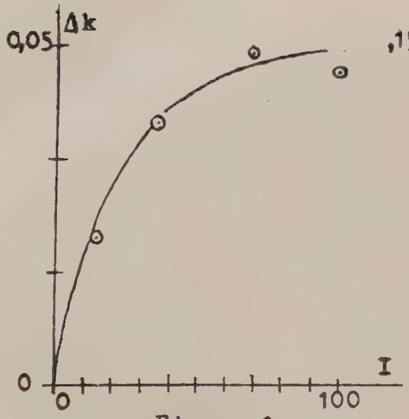

Figure 1.

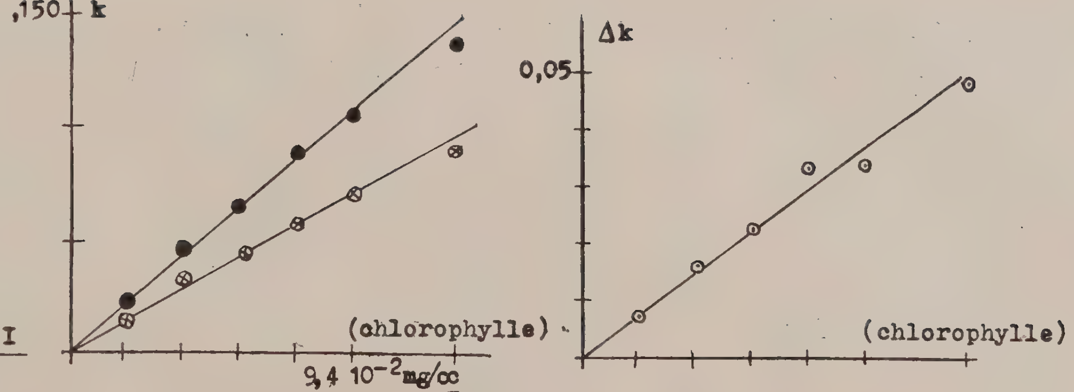

Figure 2.

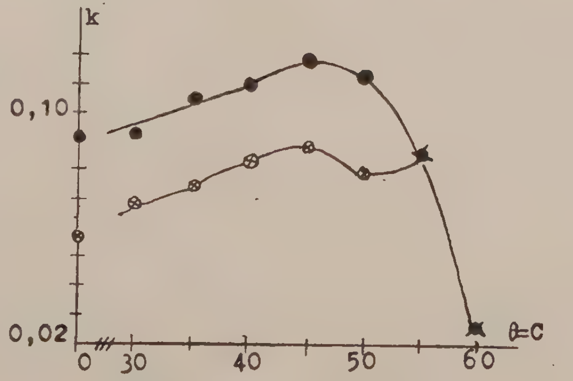

Figure 3.

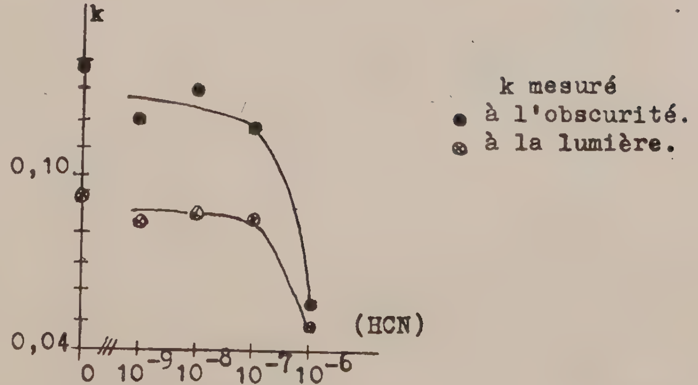

Figure 4.

donne une idée des conditions d'illumination existant dans la cuve d'expérience.

Température. — De petites quantités de suspension de chloroplastes ont été exposées pendant 5 minutes à des températures allant de 0°C à 60°C, puis l'activité catalasique à l'obscurité et à la lumière mesurée (fig. 3). L'évolution des deux activités reste sensiblement parallèle : pas de changement jusqu'à 30°, passage par un maximum entre 30 et 50°, baisse considérable d'activité et perte totale de l'effet à partir de 50°.

Cyanure. — Après incubation des chloroplastes avec la solution de HCN de concentration voulue pendant 5 minutes, l'expérience démarrait par addition rapide d'H_2O_2. Là aussi, l'effet disparaît brusquement, en même temps que se produit une forte baisse de l'activité. A 5.10^{-6} M d'HCN, l'effet est complètement nul (fig. 4).

Réversibilité. — En raison des limites matériellement imposées par la méthode, il ne m'a pas été encore possible d'étudier à fond le phénomène dans des conditions d'alternances répétées lumière-obscurité. Néanmoins, j'ai fait un certain nombre d'expériences au cours desquelles les chloroplastes étaient irradiés 5 minutes puis mis à l'obscurité 5 autres minutes, ou inversement. Il en résulte que l'alternance lumière-obscurité correspond à une vitesse moyenne de décomposition de H_2O_2 moins rapide que l'alternance obscurité-lumière. Tout se passe comme si l'action de la lumière se prolongeait après son interruption.

En conclusion, on peut faire deux ordres de remarques :

a) Cet effet est en relation avec les propriétés photochimiques des chloroplastes. Il est en effet raisonnable de penser que la lumière agit par l'intermédiaire de la chlorophylle présente à une concentration molaire environ 10^7 fois plus grande que la catalase (2), dont l'absorption est faible dans le visible. La saturation de l'effet pour les fortes intensités, dont l'analogue est classique en photosynthèse, renforce encore cette manière de voir.

b) Il ne s'agit certainement pas d'une réaction antagoniste à la décomposition enzymatique de H_20_2 comme le suggérerait le travail de MEHLER (3) où l'on peut, dans certaines conditions, mettre en évidence la réduction photochimique par les chloroplastes de O_2 en H_2O_2. Il s'agit plutôt d'une action — d'ailleurs indirecte — sur la catalase, probablement par combinaison d'un intermédiaire de la réaction de Hill avec la catalase (ce serait un peroxyde) ou avec le complexe catalase-H_2O_2 (ce serait un intermédiaire réduit). Il est facile de voir qu'il en résulterait une diminution de la constante de vitesse de la décomposition de H_2O_2. Cette interprétation est fortement suggérée par l'influence du cyanure et de la température. Enfin, s'il y a combinaison, les résultats donnés sous les rubriques « Concentration en chloroplastes » et « Réversibilité » sont en faveur d'une certaine stabilité du complexe.

(1) Sumner (J. B.) and Myrback (K.). The Enzymes, vol. II, part. I, *Acad. Press, Inc.* New-York (1951). — (2) Rabinowitch (E. I.). Photosynthesis and related processes, vol. I, *Interscience Pub.*, New-York (1945). — (3) Mehler (A. H.). *Arch. Biochem. Biophys.*, XXXIII, 65 (1951).

SOME DATA AND REMARKS ON THE STRUCTURE OF THE CHLOROPLAST

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The occurrence of chlorophyll is restricted to certain organells. In green plants they are called chloroplasts. In blue-green algae as well as in photosynthesizing bacteria these bodies are absent. Here, the chlorophylls are concentrated in less complicated structures, the grana.

The ability to photosynthesize does not necessarily depend on the presence of chloroplasts ; the blue-green algae are capable of this process as well. So one may ask whether there is some structure in common in both grana and chloroplasts. According to various authors the electron microscope revealed the following details.

Grana of blue algae and bacteria are composed of lamellae consisting of proteins or lipoproteins. They are arranged in a pile. Possibly they may alternate with lipoid layers. It seems likely that these piles are surrounded by a membrane.

The occurrence of form birefringence in chloroplasts noticed by Menke and Frey-Wyssling, indicates that lamellate structures should be envisaged in chloroplasts as well. Under the electron microscope their occurrence was observed without doubt. Electron micrographs of cross-sections of various chloroplasts, a. o. prepared by Steinmann and Wolken and Palade, showed that « chloroplasts » either may be composed of lamellae or may contain lamellate grana, *cf.* also Frey-Wyssling and Mühlethaler.

In the latter case the occurrence of chlorophyll seems to be restricted to the grana, and, most likely, in particular to their lamellae. These grana, even fragments of the lamellae, are capable of performing the Hill reaction, presumably the first photochemical step in the photosynthesis process. In structural as well as functional respect these grana may be identical with those of chloroplast-free organisms.

If, on the other hand, one compares a single granum of blue-green algae or bacteria with a lamellate « chloroplast » of e. g. *Euglena* one is quite impressed by the obvious similarity of both organells except for their size. The shape of e. g. the *Mougeotia* and *Spirogyra* « chloroplasts » differs from that of the mentioned grana. In general, however, their lamellate structure is the same. In the latter « chloroplasts » pyrenoids occur. These structures are not found in « free » grana. But, since pyrenoids seem to represent no indispensable substructures of chloroplasts, this difference may not be an essential one.

For the above reasons it is tempting to suggest that the so-called « lamellate chloroplasts » may be denoted as grana too. The grana-bearing chloroplasts or « granulated chloroplasts » may be considered to be quite different structures : they represent organells in which grana are concentrated. So it seems advisable to designate them in a different way. For these bodies the term « chloroplasts » may be retained exclusively.

2. *METABOLISME DES ACIDES ORGANIQUES*

RAPPORT

THE METABOLISM OF ORGANIC ACIDS AND CELLULAR OXIDATIONS

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The central theme in this introductory statement will be the consideration of the parts played by organic acids in the complete aerobic oxidation of carbohydrate by the cells of higher plants. This so-called respiratory oxidation is an integrated nexus of partial reactions abstracted from the total aerobic metabolism of cells. Without ambiguity, the gas-exchange quotient (CO_2/O_2) for this process, considered in isolation, may be called the respiratory quotient. The value of this quotient is unity. The gas exchange quotient can become very different from the respiratory quotient when certain collateral processes, occasioning the accumulation or disappearance of organic acids involved in respiration, accompany the complete oxidation of carbohydrate.

Treatment of our topic in this introductory paper will be strictly circumscribed. Although interesting work has been done on algae and fungi attention will be confined to higher plants. Only pyruvate and the acids in the Krebs cycle (see below) will be considered ; and, for the most part, even these acids will be viewed from a single aspect. No mention will be made, for example, of food sources of these acids other than carbohydrates, of possible alternative methods of formation from carbohydrates, or of the anabolic consumption of these acids in the synthesis of amino acids, etc. In a recent article (*Pharmazie,* 1953, vol. 8) on the biogenesis and fate of organic acids, Dr. J. Wolf gives 577 citations in the bibliography, mostly of papers which have appeared during the last five years. Clearly time alone will limit the enlargement of the subject matter during the remainder of the present colloquium.

Twenty years ago animal biochemists thought that di- and tricarboxylic acids, apart from succinic acid, were of no physiological importance, and these acids were scarcely mentioned in writings on bacterial metabolism. Little general interest was displayed in the pioneering investigations by botanists on the metabolism of vegetable acids in higher plants and mould fungi. Biochemists in general had, however, accepted the view, first put forward by botanists, that the aerobic consumption of carbohydrates involves the action of oxidation enzymes on a product or products of glycolysis. Gradually the partial reactions in phosphorylative glycolysis were elucidated, and, later, by work on animal cells and microorganisms, it was established that pyruvic acid, an intermediate metabolite in this process can be completely oxidized by way of what is called the tricarboxylic acid or citric acid or Krebs cycle. Views concerning details have changed from time to time, and finality may not yet have been reached. A brief dogmatic statement on the sequence of metabolic events, as it is pictured in early 1954, will now be made.

The evidence indicates that, acted on by pyruvic oxidase, in the presence of cocarboxylase, magnesium ions, α-lipoic acid and DPN, pyruvic acid undergoes continuous aerobic oxidative decarboxylation to yield a product which combines with coenzyme A. The resulting compound, richly endowed with energy, is a key compound in metabolism. It is called acetyl coenzyme A. The production of an acetyl radical from a molecule of pyruvate involves the absorption of one atom of oxygen and the evolution of one molecule of carbon dioxide. Condensing enzyme or citrogenase then governs the combination of this acetyl radical with oxaloacetate, which is continually being produced in the cell. Citrate is synthesized by this condensation. Entrance into the Krebs cycle has been effected. By successive enzymic step reactions, citrate is converted into oxaloacetate, which can then combine with another acetyl radical derived from pyruvate. The intermediate metabolites in this conversion are *cis*-aconitate, isocitrate, oxalosuccinate, α-ketoglutarate (succinyl coenzyme A, probably), succinate, fumarate, and malate. Two of the reactions yield carbon dioxide, one being an α-and the other a β-decarboxylation. Four of the reactions are dehydrogenations, one of which involves cytochrome C and three involve DPN or TPN as hydrogen acceptors. The regeneration of these acceptors from their reduced forms is governed by terminal oxidases. These enzymes act with oxygen, one atom of which is absorbed for each regeneration.

These views provide a satisfactory explanation of the complete oxidation of carbohydrate with a respiratory quotient of unity. The continuous aerobic production of pyruvate from carbohydrate involves oxygen uptake in the oxidation of phosphoglyceric aldehyde into phosphoglyceric acid. Carbon dioxide is not produced.

$$1/2\ C_6H_{12}O_6 + O = C_3H_4O_3 + H_2O$$

The overall equation for the complete oxidation of pyruvate is

$$C_3H_4O_3 + 5\ O = 3\ CO_2 + 2\ H_2O.$$

This equation is in accord with the facts stated above about oxygen absorption and CO_2 production relating to partial reactions which cause the entrance into the cycle and to those which occur during its operation.

The functions of the whole respiratory process comprise at least the sum of the functions of the individual partial reactions. Many of these reactions, for example the production of an acid by the dehydrogenation of an aldehyde, and the decarboxylation of a keto acid, are exergonic and lead to the synthesis of energy-rich ATP. The linkage of these partial reactions with phosphorylations appears to be an essential feature in the control respiratory oxidation exerts on growth and many cellular events. When the linkage is effective, as many as 40 molecules of ATP may be synthesized for every molecule of carbohydrate which is completely oxidized.

All these views were developed as a result of diverse experiments performed exclusively on the cells of animals and microorganisms, and on enzymes extracted from these cells. Fairly recently it has been shown that particulate masses of cytoplasm, possessing properties of mitochondria, have the power to oxidize completely pyruvate and acids of this cycle under aerobic conditions. The inference drawn is that these cytoplasmic particles contain the whole complex of enzymes and coenzymes involved in the entrance into the cycle, in each partial reaction in the cycle, and in each step of the side reactions leading to oxygen uptake by terminal oxidases. This whole complex has been called the cyclophorase system.

It is important to realize that the main facts about the mediation of the Krebs cycle in the respiratory oxidation of carbohydrate by animal cells had been well established before investigations in this field were begun on higher plants. In general the problem of determining whether or not the cycle operates in higher plants was attacked by methods which had previously proved effective in experiments on the cells of animals and microorganisms. Information about higher plants has been acquired relatively slowly, and there are still wide gaps in our knowledge, some of which may be brought into prominence in the present colloquium. For example, at the time of writing (January 1954) one may ask whether there is any direct evidence that acetyl coenzyme A is a key substance in the metabolism of higher plants ? Nevertheless, convincing evidence has been amassed of the occurrence of reactions of the Krebs cycle in higher plants.

A significant point is that, when searched for by chromatographic or older methods, some groups of acids which participate in the Krebs cycle in animal cells have been found to exist together in plant cells. Of course plant cells differ from animal cells in that they often contain noteworthy accumulations of some of these acids, signifying a production rate which greatly exceeds the consumption rate. Enzymic and metabolic conversions have been investigated. The presence in higher plants of many of the enzymes and coenzymes governing the partial reactions in the cycle has now been detected. However, the higher plant has not proved good material for the preparation of purified specimens. The power of cells to consume oxidatively pyruvate and acids in the cycle has been demonstrated by feeding experiments of various kinds. Particulate cytoplasmic material of a mitochondrial type, possessing similar powers, has been separated from mung bean and pea seedlings. Lactic acid, which accumulates in potato tubers placed under anaerobic conditions, is oxidatively consumed on re-exposure of the tubers to air. Evidence has been obtained that consumption is by way of pyruvtae and keto acids in the cycle. Chromatographic methods have facilitated the demonstration of conversions involving one or more partial reactions in the cycle by cells and mitochondria.

The fate of some acids containing labelled carbon has been determined. In general when inhibitors have been added to the reacting systems, predictions based on the postulate that products of glycolysis are oxidized by way of the cycle have been fulfilled. For example, malonate has been effectively used in repeating for plants Krebs's key experiments on the conversion by animal cells of pyruvate to succinate *via* citrate. More recently it has been shown that malonate inhibition may result in the production of ethyl alcohol and acetaldehyde (malonate zymasis). Gas exchange quotients have been measured during the accumulation or consumption of acids in the presence and absence of inhibitors. Some of the values obtained have been explained reasonably well. To explain certain excessively high values during the disappearance of acids, we may have to wait for fuller knowledge of the extent to which acids may be consumed by conversion into carbohydrate as well as by oxidation.

The maintenance of the cycle in operation clearly requires a continuous supply of oxaloacetate. It can be argued that if this supply becomes limiting whilst glycolysis is vigorous, pyruvate will tend to accumulate and be converted into lactic acid, acetaldehyde, ethyl alcohol, and possibly other so-called fermentation products. Normally, plant cells living in air do not contain appreciable amounts of such fermentation products. One might infer that the rate of production of oxaloacetate is normally adequate to prevent the accumulation of pyruvate in spite of the fact that a reduction in the potential amount of oxaloacetate in the cycle will result from every side reaction (e. g. the formation of an amino acid) which removes a member of the cycle. In many plant organs there is an ample reservoir of accumulated acids from which oxaloacetate can be produced.

In addition, in experiments with $C^{14}O_2$ it has been shown, for a number of roots and shoot organs, that the store of dicarboxylic acids can be augmented by the β-carboxylation of pyruvate under the agency of oxaloacetate β-carboxylase in the Wood-Werkman reaction, or under that of Ochoa's malic enzyme. Dark CO_2 fixation can also take place by the β-carboxylation of α-ketoglutaric acid to yield oxalosuccinate (the Ochoa reaction). It may well be that β-carboxylation has high functional value during the developmental phase in all plant cells, and, also, in those mature cells in which the amount of accumulated acid is low. It is possible that β-carboxylation is a superfluous event in cells richly stocked with malic, citric, isocitric or some other acid belonging to the cycle, and therefore a potential source of oxaloacetic acid. Of course the process may have some other function which does not relate to oxidative metabolism. It is at least clear that it does no harm.

In plant organs which show crassulacean acid metabolism extensive malic acid accumulation occurs in the dark owing to the fixation of respiratory carbon dioxide and of such amounts of carbon dioxide as are absorbed from the environment. As a result of this fixation the gas exchange quotient for organs metabolizing in CO_2 free air may fall to zero. When carbon dioxide is present in the environment negative values for the quotient have been found during dark acidification. Clearly these plants are well suited for carrying out investigations on the physiological conditions which affect β-carboxylation *in vivo*. The process is readily followed without using carbon dioxide containing labelled carbon.

Among the external factors which have been investigated are, temperature, oxygen concentration, CO_2 concentrations at low and high levels, and a number of well-known enzyme inhibitors. The amount of dark acidification increases as the temperature is lowered, although the initial rate may be slower. *In vivo,* β-carboxylation appears to come to a standstill very soon in the complete absence of oxygen, but it occurs appreciably in concentrations less than 1 per cent, and continues until cells are injured by causes relating to oxygen want. Raising the oxygen concentration promotes dark acidification ; the effects of increases at the lower levels of oxygen concentration are relatively great. These effects have not yet been satisfactorily explained. Increase in external CO_2 concentration up to some value which lies between 1 to 5 per cent may promote dark acidification. Possibly carbon dioxide is acting as a metabolite in limiting concentration. At CO_2 concentrations exceeding 5 per cent the β-carboxylation system may become saturated, but retain unimpaired activity. At higher CO_2 concentrations the enzyme or enzymes involved are inhibited. Inhibitory effects have been observed in the range 15-30 per cent. Complete inhibition has been brought about by 50 per cent carbon dioxide. Cyanide at low concentrations is one of the chemical poisons which can suppress dark acidification. Inhibitions may be accompanied by the production of ethyl alcohol (*i. e.* by zymasis) as a consequential or parallel phenomenon.

It remains to be determined whether or not a special functional value attaches to the remarkable power of β-carboxylation possessed in the summer by plants showing crassulacean acid metabolism. Such evidence as is available indicates that dark acidification is a process additional to and not a substitute for the normal type of « respiratory » oxidation of carbohydrate with which this paper is mainly concerned. Indeed it may be held as a working hypothesis that a feature common to all higher plants is that the Krebs cycle operates in the metabolism of organic acid in cellular oxidations. (I again emphasize that I am intentionally disregarding the possible occurrence of other oxidative cycles or sequences which involve acids.)

Many quantitative modifications may result because partial reactions relating to the Krebs cycle proceed at different relative rates in different plants, or in a given plant in different tissues, or in a given tissue at different stages of development, or in a given tissue in a given state of development under different environmental conditions ; but underlying all these modifications is a complete oxidation of a fraction of all the carbohydrate consumed. The gas exchange quotient for the total metabolism of carbohydrate may be very variable, but for the oxidation of this « respired » fraction the value of the quotient (CO_2 produced but not necessarily evolved/oxygen absorbed), *i. e.* the real « respiratory » quotient for carbohydrate, is unity. Extensive further work on higher plants is required to test the hypothesis and its consequences. As yet few wide generalizations can be made with assurance.

EVOLUTION DES ACIDES ORGANIQUES CHEZ LA VIGNE

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Des dosages d'acide malique, faits du 16 juin au 8 octobre 1951, sur les trois principaux cépages du Bordelais : Sémillon (blanc), Merlot et Cabernet-Sauvignon (rouge), ont révélé une accumulation importante d'acide malique dans les feuilles, au fur et à mesure que la végétation progresse. Les dosages étaient pratiqués toujours sur les troisième et quatrième feuilles des rameaux fructifères, à partir de la base ; les chiffres donnent donc l'enrichissement progressif d'une feuille développée au printemps ; les prises d'échantillons étaient de l'ordre du kilogramme, prélevés sur plusieurs centaines de pieds, au petit jour et à la tombée de la nuit ; aucune différence systématique n'a été observée entre les deux sortes de prélèvements. Le chiffre pour une date donnée a donc la valeur d'une moyenne pour le cépage et le vignoble donné (Léognan, au sud de Bordeaux). Les teneurs en acide malique, faibles à la mi-juin (42 à 58 milliéquivalents par kg. de feuille fraîche) augmentent jusqu'à un maximum de 150 à 200 m.éq., fin août pour Merlot et Sémillon, fin septembre pour Cabernet-Sauvignon.

Dans la pulpe de la baie, les taux d'acide malique sont généralement inférieurs aux taux dans les feuilles ; toutefois, fin août, il y a plus d'acide malique dans la baie que dans la feuille. Dans la pellicule de la baie (l'épiderme et les couches parenchymateuses mécaniquement liées), l'acide malique est à un taux relativement constant, de l'ordre de 100 m. éq. par kg. frais. Dans la pulpe, durant le mois de septembre, l'acide malique disparaît pour les trois-quarts, tandis que le sucre s'accumule ; voici les chiffres du 25 août et du 8 octobre 1951 : Sémillon 342 et 85, Merlot 332 et 72, Sauvignon 316 et 79, Cabernet-Sauvignon 250 et 90. L'acide tartrique décroît un peu moins : Sémillon 139 et 69, Merlot 140 et 57, Cabernet-Sauvignon 135 et 54. Dans les pellicules, l'acide tartrique décroît : Sémillon 85 à 64 ; ou croît : Sauvignon 78 et 99, Merlot 65 et 80, Cabernet-Sauvignon 68 et 79.

Tout se passe comme si la masse principale des acides organiques était élaborée par la feuille, émigrait vers la baie en juillet-août ; le catabolisme respiratoire fait disparaître de la baie une quantité importante de ces acides durant septembre, lorsque le sucre s'accumule ; ces faits ont déjà été partiellement décrits par E. Peynaud (1938-1947) et sur d'autres cépages par Laure Gatet (1940).

Les pellicules se distinguent de la pulpe par un taux très élevé de salification des acides organiques : l'alcalinité des cendres y atteint 75 à 120 m.éq. par kg., pour 60 à 100 d'acides libres ; dans la pulpe, on observe 30 à 45 d'alcalinité pour 100 à 500 d'acides libres ; l'alcalinité des pellicules croît rapidement durant les dernières semaines de maturation, celle de la pulpe variant peu. Il y a donc migration considérable de cations minéraux vers la pellicule, qui se comporte comme une feuille.

La maturation du raisin a été suivie en 1952 dans cinq domaines différents, distants au total de 100 km. environ, au nord et au sud de Bordeaux. Le microclimat détermine, dans un même cépage, Merlot, des taux d'acide malique très différents : le 28 juillet 1952, on notait (m.éq. par litre de jus) : 278, 266, 224, 210, 136 ; le 15 septembre 1952, ces différences ont presque disparu : 48, 34, 38, 29, 32, taux qui ne varient plus sensiblement, qui sont presque moitié moindres que les taux de 1951, chiffrant ainsi la qualité exceptionnelle de la récolte de 1952. Les taux d'acide tartrique sont, dans le même ordre des stations, au 28 juillet, 217, 246, 300, 231, 217 ; au 15 septembre, 97, 95, 113, 100, 91, taux plus élevés qu'en 1951. Le Cabernet-Sauvignon, étudié dans les mêmes stations, a montré le même comportement.

NOTE SUR LES ACIDES FORMES AU COURS DE LA GERMINATION DU RICIN

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Les acides organiques constituant incontestablement des substances communes aux métabolismes des glucides, lipides et protides, nous avons recherché quels étaient les acides formés au cours de la germination du Ricin, acides gras proprement dits exceptés.

Sans préjuger ici du rôle joué par les divers acides, nous indiquons sommairement les techniques utilisées et les résultats obtenus. Leurs propriétés physiques et chimiques permettent de les séparer en trois groupes : acides volatils, acides fixes stables, acides cétoniques. Chacun des deux premiers groupes est fractionné en ses constituants par passage sur colonne de silice ; le troisième est précipité sous forme de 2-4 dinitrophénylhydrazones. Les identifications sont faites dans chaque cas par chromatographie sur papier.

Nous avons ainsi pu identifier dans le premier groupe les acides formique, acétique et butyrique ; dans le second, les acides glycolique, lactique, succinique, fumarique, malique et citrique ; dans le troisième, les acides pyruvique, α-cétoglutarique et ascorbique. Dans ce dernier groupe nous avons trois autres acides qui, pour l'instant, ne sont pas encore déterminés. Les proportions relatives de ces divers acides ne sont pas les mêmes dans les plantules et dans l'albumen et varient avec l'âge.

ACID METABOLISM IN ATMOSPHERES WHICH INHIBIT DARK CO_2 — FIXATION

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The ability to synthesize dicarboxylic acids by dark CO_2 fixation under the agency of β-carboxylases has been demonstrated in many higher plants and may well be an attribute of all plant cells. In plants showing Crassulacean acid metabolism the reaction is of such magnitude that it is easily demonstrated by measurement of gaseous exchanges in CO_2-enriched atmospheres. For example, 100 g. of *Kalanchoe* leaves have fixed 250 mg. of CO_2 in 15 hours. In most other plants the reaction is comparatively slow and its demonstration has required the use of labelled CO_2. Relative rates of carbon-14 incorporation in one experiment with more or less mature tissues were : *Kalanchoe,* 100 ; green apples and oat coleoptiles, 1 ; gooseberries and rhubarb leaves, 0.5 ; rhubarb petioles and green grapes, 0,2. However, it is possible that the fixation rates may vary considerably during the different stages of development. In all tissues more of the ^{14}C accumulated in malic acid than in any other single component, suggesting a similar fixation reaction in each case. The functions of this reaction, other than possibly the maintenance of a supply of oxalacetate as an acceptor of acetyl radicals, are unknown.

In *Kalanchoe* the CO_2-fixation is markedly inhibited in CO_2 concentrations above about 50 % and in oxygen concentrations below about 1 %. In these gas mixtures most plants accumulate ethyl alcohol (or lactic acid). This suggested that inhibition of CO_2-fixation by leading to a depletion of oxalacetate might cause glycolysis products to be diverted from the oxidative acid cycle to the formation of alcohol. This simple hypothesis is inadequate for tissues, such as apple, which contain large acid reserves, unless (a) one or more of the partial reactions relating to the Krebs acid cycle are simultaneously inhibited, or (b) the bulk of the accumulated acid is spatially remote from, or for other reasons is equilibrated slowly with, acid reservoirs of the Krebs cycle.

To investigate the problem a variety of tissues have been analysed chromatographically after periods of storage in the inhibiting gas mixtures. In high CO_2 concentrations malate is depleted and succinate accumulates. In low oxygen concentrations succinate accumulates but there is no equivalent depletion of malate. In both cases there are concomitant changes in amino and keto acid concentrations. These changes in acid metabolism cannot yet be explained but preliminary experiments suggest that succinate may be formed in reactions independent of those in the Krebs acid cycle.

FORMATION OF DICARBOXYLIC ACIDS BY CO_2 FIXATION

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There are at least three short enzymic routes whereby CO_2 carbon may be incorporated into malate or oxalacetate in plant tissues. One involves the action of the malic enzyme of Ochoa. A second involves the exchange reaction of CO_2, into the β-carboxyl group of oxalacetate in the presence of adenosine triphosphate, and is similar to the reaction studied by Utter and Kurahashi in pigeon liver. A third involves a condensation of phosphopyruvate and CO_2 as described by Bandurski and Greiner for spinach leaves. The third reaction, unlike the second, is independent of any nucleotides. All of these three reactions occur simultaneously in extracts of wheat germ. The mechanism and the metabolic significance of these reactions will be discussed, with special reference to their interdependence.

LES ACIDES DES FEUILLES ET FRUITS DES ROSACEES

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La technique de chromatographie sur papier des acides organiques, mise au point par Cheftel, Machebœuf et Munier, nous a permis une étude qualitative des acides organiques des feuilles et des fruits chez les Rosacées cultivées. Les acides étaient extraits du végétal à l'eau bouillante, précipités sous forme de sels de Pb, mis en liberté par HCl après élimination du Pb sous forme de SO_4Pb, desséchés, repris par la pyridine anhydre, desséchés à nouveau, repris par l'eau distillée. Cette solution aqueuse était alors chromatographiée sur papier au moyen du butanol formique. Appliquée à la vigne, cette technique a permis de retrouver les résultats connus : acides malique et tartrique sont les principaux acides, accompagnés d'un peu d'acide citrique ; dans les feuilles et les fruits verts, de petites quantités d'acides indéterminés.

Appliquée aux Rosacées, cette technique a donné des résultats surprenants : les feuilles renferment des acides indéterminés, généralement plus

importants que les acides citrique et malique. Les Rf de ces acides sont voisins de ceux des esters phosphoriques des oses ; certains contiennent effectivement de l'acide phosphorique. Les fruits, à noyaux ou à pépins, renferment aussi des acides inconnus, mais les acides malique, et parfois citrique, sont prédominants, surtout dans les fruits mûrs.

Fruits à noyau.

Cerisiers, Belle-d'Orléans, Impératrice-Eugénie, prunier Reine-Claude hâtive, Bonne-de-Bry. Feuilles : des acides indéterminés, quelquefois accompagnés d'acides malique et citrique. Fruits : de l'acide malique, qui diminue au cours de la maturation, des acides indéterminés.

Pêchers, May Flower, Amsden. Feuilles : acides indéterminés, de petites quantités des acides malique et citrique. Fruits : de l'acide malique, de l'acide citrique, qui disparaissent partiellement à la maturation ; des acides indéterminés.

Abricotier Bruhel. Feuilles : acides indéterminés. Fruits : acides malique et citrique du même ordre, l'acide citrique venant plus tardivement. Fruits verts : traces d'acides inconnus.

Fruits à pépins.

Poirier. Marguerite-Marillat, Sainte-Anne. Feuilles : un peu d'acides indéterminés ; des quantités notables d'acide malique, un peu d'acide citrique. Fruits : acide malique prédominant, parfois acide citrique ; quelques acides inconnus.

Fraisier hâtif. Feuilles : acides indéterminés. Fruits : acide citrique prédominant, un peu d'acide malique ; acides inconnus disparaissant dans le fruit mûr.

Groseillier rouge hâtif de Berlin. Feuilles : acides indéterminés, parfois un peu d'acide citrique. Fruits : acide citrique prédominant, diminuant à maturité ; des acides inconnus dans le fruit vert.

LES ACIDES CITRIQUE ET MALIQUE DES ALGUES MARINES

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Dans une première série de recherches (1952) portant sur une cinquantaine d'espèces, il a été démontré que l'acide citrique était un constituant normal des Algues marines.

On s'est attaché depuis à étudier la répartition des acides citrique et malique dans les mêmes tissus de diverses Algues marines.

Les chiffres cités ci-dessous sont extraits d'un ensemble provenant de l'examen d'une vingtaine d'espèces récoltées en janvier sur la Côte basque (+) et près de Brest au mois de mars 1953.

La teneur en eau variant de 94 à 72,5 % selon les tissus considérés, on a préféré exprimer les résultats par rapport au poids sec.

Voici les teneurs en acides citrique, malique et alcalinité des cendres, exprimées en milliéquivalents par kg. de tissu sec :

Chlorophycées : *Codium tomentosum* (+), 3,4 — 3,3 — 1.073 ; *Ulva lactuca*, 4 — 107 — 1.387.

Phéophycées : *Fucus vesiculosus* ♀, Thalle 301 — 73 — 1.324 ; Réceptacles mûrs 180 — 40 — 891 ; *Fucus serratus* ♂, Thalle 140 — 56 — 2.150 ; Réceptacles mûrs 104 — 23 — 1.341 ; *F. serratus* ♀, Thalle 68 — 34 — 650 ; Réceptacles mûrs 112 — 42 — 1.215 ; *Ascophyllum nodosum*, Thalle 212 — 37 — 1487 ; Réceptacles immatures 79 — 28 — 1.231 ; Réceptacles mûrs 435 — 39 — 1.439 ; *Cystoseira ericoides* (+), Thalle 14 — 5 — 1.720 ; *Laminaria digitata*, Stipe 16 — 37 — 2.480 ; Base de la Fronde 14 — 30 — 1.420.

Rhodophycées : *Pterocladia capillacea* (+), Thalle 6,4 — 7,3 — 682 ; *Chondrus crispus*, Thalle 24 — 103 — 609 ; *Rhodymenia palmata*, Thalle 27 — 52 — 420.

Dans la limite des recherches effectuées, l'acide malique est donc lui aussi un élément normal des tissus des Algues marines.

Tout comme chez les Phanérogames, la répartition des acides citrique et malique est ici loin d'être uniforme.

DER IONENAUSTAUSCH BEI SPHAGNUM UND DIE ENTSTEHUNG DER MOORSAEUREN

von Fritz Gessner

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Die Säurewirkung der Torfmoose (*Sphagnum*) ist ein Problem, das in den letzten 50 Jahren verschiedenste Deutungen gefunden hat ohne dass dadurch eine wirkliche Klärung erreicht worden wäre.

Es lässt sich nun leicht zeigen, dass die Säurebildung auf dem Wege eines Ionenaustausches stattfindet, wobei das *Sphagnum* im lebenden oder im toten Zustand als Kationenaustauscher wirkt.

Dass es sich um eine Austauschwirkung handelt, lässt sich schon daraus erkennen, dass sorgfältig gereinigtes Sphagnum in destilliertem Wasser keine pH-Senkung herbeiführt, während sich der pH-Wert in Gegenwart von Neutralsalzen innerhalb weniger Sekunden auf 4 senkt, wenn *Sphagnum* mit der Lösung in Berührung kommt. Hierbei belädt sich das

Sphagnum mit Metallionen und gibt hiefür die äquivalente Menge an H-Ionen an die Lösung ab.

Bei *Sphagnum* im natürlichen Zustand hat dieser Austausch bis zu einem gewissen Grade bereits stattgefunden, so dass die Moose nicht mehr über ihre maximale H-Ionenaustauschkapazität verfügen. Wie bei den künstlichen, in der Technik verwendeten Harzaustauschern lässt sich das Material jedoch « regenerieren, d, h., bei sehr höhen H-Ionenkonzentrationen findet ein Rücktausch der H-Ionen » gegen Abgabe der Metallionen statt. Bei einem auf diese Weise regenerierten *Sphagnum* kann einerseits geprüft werden, ob der Ionenaustausch im stöchiometrischen Verhältnis erfolgt, andererseits kann die maximale Austauschkapazität bestimmt werden.

Wird eine bestimmte Menge *Sphagnum* in eine 10 % Lösung von Bariumchlorid gelegt, so wird für die aufgenommenen Bariumionen die äquivalente Menge an H-Ionen abgegeben. In konzentrierten Säuren können diese Barium-Ionen wieder quantitativ aus dem *Sphagnum* zurückgewonnen werden, wobei sich das Torfmoos maximal mit H-Ionen aufladet.

Mit diesem Versuch ist der Beweis erbracht, dass es sich wirklich-und zwar auch im quantitativen Sinne-um einen Ionenaustausch handelt.

Es können somit unsere Erfahrungen, die an Harzaustauschern gewonnen wurden, auf das *Sphagnum* angewendet werden. Allerdings ist das *Sphagnum* in diesem Sinne ein schlechter Austauscher, da das Gleichgewicht schon etwa bei pH 4 erreicht wird und bei niedrigeren pH-Werten kein Austausch, bzw. sogar ein Rücktausch stattfindet.

Wie bei den Harzaustauschern ist auch bei *Sphagnum* die Neutralsatzaustauschkapazität bei zweiwertigen Ionen grösser als bei einwertigen, wie folgende Tabelle zeigt :

Neutralsalzaustauschkapazität in Milliäquivalenten pro 100 g trockenes *Sphagnum cuspidatum* in 0.1 n Lösungen

Li Cl	25.2	$MgCl_2$	34.6
NaCl	19.1	$CaCl_2$	33.6
K CL	20.4	$BaCl_2$	37.9

Zum Ionenaustausch ist lebendes und totes *Sphagnum* in gleicher Weise befähigt. Mit der Dauer der trockenen Aufbewahrung nimmt allerdings die Fähigkeit zum Ionenaustausch im Laufe einiger Jahrzehnte ab und nach ca 70 Jahren ist keine Säurewirkung mehr festzustellen.

Von der Temperatur ist der Vorgang des Ionenaustausches weitgehend unabhängig, wie dies auch bei den Harzaustauschern der Fall ist. Die einzelnen *Sphagnum*-Arten unterschieden sich wesentlich in ihrer Austauschkapazität ; am grössten ist sie bei *Sph. palustre.*

Der Chemismus der austauschenden Substanz im *Sphagnum* konnte bisher nicht aufgeklärt werden. Aus der Neutralisationskurve kann geschlos-

sen werden, dass COOH die wirksame Gruppe ist, doch ist die Beteiligung anderer Gruppen am Austauschvorgang wahrscheinlich.

Résumé. — Les *Sphagnum* vivants ou morts se comportent comme des échangeurs de cations. Dans une solution saline, ils fixent les ions métalliques et libèrent une quantité équivalente d'ions H^+. On peut ensuite « régénérer » ces *Sphagnum* comme des échangeurs d'ions de laboratoires. Les échanges sont indépendants de la température, mais la capacité varie d'une espèce de *Sphagnum* à une autre. Les carboxyles -COOH du *Sphagnum* jouent un rôle important dans ces échanges.

METABOLISME DES SUBSTANCES AZOTEES

THE SYNTHESIS AND INTERCONVERSION OF AMINO-ACIDS IN BARLEY SEEDLINGS

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The metabolism of amino-acids during the early phases of seed germination is mainly concerned with the transformation of the reserve proteins of the storage tissues. In the young seedling a rapid synthesis of the structural proteins of the protoplasm occurs at the expense of the reserve proteins. It has been shown that in barley, germinated under favourable conditions, nearly all the reserve proteins of the endosperm are mobilised during the first 8-10 days of germination and transferred to the embryo, where active synthesis of tissue proteins occurs. There is already some evidence that the transformation of reserve to protoplasmic proteins involves an extensive interconversion of amino-acids. This evidence arises from the fact that the constituent amino-acids of the reserve proteins differ widely from those of the cytoplasmic proteins. One of the chief objectives of the work described here was to obtain a more exact account of the overall changes of amino-acids which take place during germination.

For an analysis of the changes underlying the metabolism of proteins in the seedlings a more complete knowledge of the constituent amino-acids of both reserve and tissue proteins was necessary. With this object preparations of the endospermic proteins were made and analysed mainly by means of microbiological assay. Estimates of eighteen amino-acids were obtained and these, together with amide-N, accounted for 90-100 % of the protein-N. The two main reserve proteins of the endosperm, a prolamin, hordein, and a glutelin, hordenin, are both rich in amide-N, glutamic acid and proline, but relatively deficient in basic amino-acids. Over 45 % of the nitrogen of hordein is in glutamic acid and amide-N which are in all probability combined as glutaminyl residues in the protein. Albumins and globulins are present in much smaller amounts in the endosperm and have a more balanced composition resembling that of the tissue proteins. Representative preparations of tissue proteins, including cytoplasmic and chloroplastic components, were made from young seedlings and analysed in a similar way. With regard to their constituent amino-acids, the proteins of the seedling closely resembled those prepared from mature leaves of the plant ; they differed only in having a somewhat higher proportion of lysine than the leaf proteins.

The most precise estimates of the interconversion of amino-acids can be made when seedlings are germinated without external supplies of nitrogen. Under these circumstances the reserves of the endosperm are the only source of nitrogenous nutrient available to the developing embryo. Thus analyses of the amino-acids in the protein and non-protein fractions of the embryo and endosperm provide a fairly complete account of the overall changes at different stages of germination. Experiments have been carried out in this way with several samples of grain, differing widely in their protein content, and from these data it is possible to calculate the changes in content of each amino-acid during germination.

Syntheses of aspartic acid, alanine, lysine, arginine and to a smaller extent of glycine, isoleucine, tryptophan and threonine were uniformly found. Other nitrogenous constituents showing a marked increase were purines and chlorophyll. In all of the experiments about 90 % of the nitrogen required for these syntheses was derived from amide-N, glutamic acid and proline of the reserve proteins. Small overall decreases of phenylalanine and serine were also observed consistently.

The available data indicate that glutamic acid plays an important rôle in the interconversion of amino-acids during germination of the grain. It has also been shown that this amino-acid and its amide, glutamine, are rapidly formed when nitrates and ammonium salts are supplied to roots of young seedlings and the primary synthesis of the amide under these conditions has been established by using isotopic nitrogen as a tracer. At an early stage in the development of the embryo enzymic systems are present which promote transamination and transamidation of glutamic acid and glutamine respectively, further suggesting the key position of these reactants in the synthesis of proteins in the seedling.

PROTEIN METABOLISM IN ANANAS COMOSUS

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Protein accumulation by synthesis, and depletion by hydrolysis, in the tissues of *A. comosus* is subject to change in age, nutrition and climate.

Ammonium (NH_4) versus nitrate (NO_3) nutrition changes the absolute and relative amounts of proteinaceous and non-proteinaceous compounds of the tissues. NH_4, on account of greater rates of intake and assimilation in the roots immediately following absorption, increases the N-compounds of the tissues more than NO_3. Nitrate is assimilated in the chlorophyllous leaf tissues.

In all cases the plants of *A. comosus* were grown for eleven months under the conditions specified and then subjected to chemical analysis, as reported in the original publications.

Protein accumulations are greater in the chlorophyllous than in non-chlorophyllous tissues. In the chlorophyllous tissues of mature and active leaves, protein accumulations are higher than in younger leaves. Protein accumulations in the stem are greatest in the apical meristematic tissues. Also, accumulations of soluble organic nitrogen, resulting from synthesis from inorganic-N or from protein hydrolysis in the leaves, as suggested by relatively high soluble organic-N in plants grown in darkness, are greater in the apical tissues than elsewhere in the stem.

Protein accumulations in the chlorophyllous tissues of leaves depend on the quantity and quality of inorganic nitrogen supplied to plants. For instance, leaves of plants supplied with 0.2 and 10.0 m.eq. per liter of ammonium-N contained, respectively, 0.548 and 2.240 mg of protein-N per gram of fresh tissue. Leaves of plants supplied with 0.2 and 10.0 m.eq. of nitrate-N per liter contained, respectively, 0.530 and 1.800 mg of protein-N.

Protein accumulations in the apical stem tissues differed considerably with respect to the quantities of inorganic-N. For example, the apical stem tissues of plants supplied with 0.2 and 10.0 m.eq. of nitrate-N per liter contained, respectively, 0.755 and 0.578 mg of protein-N. Similar tissues of plants with 0.2 and 10.0 m.eq of ammonium-N per liter contained, respectively, 1.365 and 0.944 mg of protein-N per gram of fresh tissue.

The greater amounts of protein-N in the stems of plants with 0.2 than with 10.0 m.eq. of NO_3 or NH_4 nitrogen per liter were presumably due to concentration effects resulting from smaller plant weights in the 0.2 m.eq. of N cultures. The weights of the 10.0 m.eq. of N cultures were 1.33 and 1.75 times as great as those of the 0.2 m.eq. of N cultures, for the ammonium-N and nitrate-N series, respectively. The protein concentrations were 1.45 and 1.30 times as great for the 0.2 as for the 10.0 m.eq. of N cultures, for the ammonium-N and nitrate-N series, respectively. The results show that the protein-N concentrations effects were not absolutely proportional to the reduction of plant weight in the 0.2 m.eq. of N cultures.

Soluble organic nitrogen accumulations in the chlorophyllous leaf tissue of the nitrate-N plants were 0.139 and 0.513, and in the ammonium-N plants 1.062 and 1.145 mg per gram of fresh tissue for the 0.2 and 10.0 m.eq. cultures, respectively. Similar accumulations in the stem tissue were, respectively, for the 0.2 and 10.0 m.eq. cultures 0.354 and 1.045 in the nitrate-N plants and 0.647 and 1.315 mg in the ammonium-N plants.

Soluble organic nitrogen concentrations, although lower in the 0.2 than in the 10.0 m.eq. cultures, were considerably higher than inorganic NO_3- or NH_4- nitrogen which were found only in traces or were lacking in the tissues. Hence, the probable sources of soluble organic nitrogen were the proteins which through continuous anabolic and catabolic changes maintained a relatively constant stream of soluble organic nitrogen in the tissues.

In plants with nitrogen deficiency, new growth is maintained, although at considerably reduced rates, by the utilization of soluble organic nitrogen released presumably by hydrolysis from the proteins of old leaf tissues.

A. comosus, grown in darkness for six weeks, contained in the apical stem tissues 0.80, and in the chorophyllous leaf tissues 0.52 times as much protein as plants exposed to sunlight. The changes in protein were reflected in soluble organic nitrogen which, in the apical stem tissues, was 4.4, and in the chlorophyllous leaf tissues 1.23 times as great in the darkened as in the exposed plants.

Iron deficiency, either by reducing the concentrations of chlorophyll or the Fe-containing oxidation-reduction activators essential in normal cell metabolism, caused protein reductions of approximately 50 % in the chlorophyllous leaf tissue and 18 % in the apical stem tissues. The rates of nitrate assimilation and, in turn, of protein synthesis were also reduced by iron deficiency.

Potassium deficiency in cultures with 0.1 vs 5.0 m.eq. per liter of K increased soluble organic-N accumulations in fresh leaf tissue of the former to 125 % and 260 %, respectively, for the NO_3' and NH_4 series.

It has been observed that under various nutritional conditions high protein correlated with high chlorophyll concentrations.

At different diurnal intervals leaf and stem protein changes phases becoming reversibly sap-soluble from sap-insoluble due to fluctuations in the acidity of the sap. These proteins are less soluble at high than at low acidity, high acidity increasing in darkness, at night, and decreasing in light, during the day.

PROTEIN SYNTHESIS IN RELATION TO CELL GROWTH

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The basic living unit is the cell. We visualize and express every morphological and anatomical development in terms of cells. Yet for biochemical and physiological data we ignore this fundamental living unit of the organism and record data in terms of weight of tissue.

For this study on protein content of cells during their division and subsequent enlargement, coleoptiles of *Avena sativa* were used. Early growth in this organ of grass seedlings is largely due to an increase in the number of cells and their accompagying enlargement, whereas later growth consists solely of cell elongation. Exact cell counts made it possible to express protein content per cell, at all stages of development.

The microchemical method was that of the Carlsberg Laboratory in Copenhagen.

Results : The greatest overall increase in protein occurs during the meristematic phase of growth, but it also continues to increase when cells are undergoing only elongation. Cell enlargement thus depends not solely on water uptake, but also on the synthesis of additional protoplasm. The protein increase during enlargement is approximately in proportion to the increase in cell volume.

3. *BIOGENESE DES SUBSTANCES SECONDAIRES*

RAPPORT

DIE BIOGENESE SEKUNDAERER PFLANZENSTOFFE

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Noch vor wenigen Jahren war über den Stoffwechsel, der zur Entstehung sekundärer Pflanzenstoffe führt, kaum etwas Sicheres zu sagen (vergl. Paech 1950). Hypothetische Vorstellungen beherrschten das Denken sowohl der Biochemiker als auch der Botaniker. Erfolgreiche Versuche wurden erst in Angriff genommen, als der allgemeine Umsatz der Kohlenhydrate genauer geklärt und einige wichtige Prinzipien des pflanzlichen Stoffwechsels, auf die hier nicht näher eingegangen zu werden braucht, aufgedeckt worden waren. Inzwischen sind schon für verschiedene Gruppen sekundärer Verbindungen experimentelle Unterlagen zu ihrer Biogenese gesammelt worden, so dass es heute fruchtbar erscheint, den Chemismus ihrer Bildung vergleichend zu betrachten.

Als sekundäre Pflanzenstoffe, deren Abgrenzung von anderen Bestandteilen der Pflanzen niemals ganz scharf sein wird, darf man diejenigen Verbindungen bezeichnen, die über das zum Leben der einfachsten Pflanzenzelle Notwendige hinaus produziert werden. Diese Stoffe können allerdings häufig Funktionen, z. B. ökologische, übernehmen und werden dadurch für die betreffende Art lebensnotwendig. Ein grosser Teil typischer sekundärer Pflanzenstoffe wird konventionell in folgende Gruppen zusammengefasst : fette Oele, Terpenoide (viele ätherische Oele, Harze, Sterine, Carotinoide, Kautschuk), Gerbstoffe, Lignin, Anthocyane und Anthoxanthine, Laktone (z. B. Cumarine), Glykoside, Alkaloide usw. Diese Einteilung, die bei weitem nicht alle hierher gehörigen Verbindungen deckt, ist praktischtechnisch oder jedenfalls recht oberflächlich und besagt nicht viel über die Eingliederung in den Stoffwechsel oder über die physiologische Bedeutung der Stoffe.

Das Problem der Genese sekundärer Stoffe hat häufig zwei wesentlich verschiedene Seiten, denn nur eine betrifft den Chemismus der Synthese aus den meist noch unbekannten Bausteinen. Die andere bezieht sich darauf, dass sekundäre Stoffe im allgemeinen nur in begrenzten Teilen der Pflanzen, in bestimmten Organen, Geweben oder nur in Einzelzellen auftreten. Häufig sind spezielle Behälter für sie ausgebilden (Oldrüsen, Harzgänge, Milchröhren), oder die sekundären Stoffe werden nur in die Wände inkrustiert oder auf der Epidermis ausgeschieden. Diese zweite Teilfrage nach den Faktoren, die gewisse Zellen oder Zellgruppen zur Bildung und Ansammlung der speziellen Substanzen veranlassen, stellt natürlich einen Ausschnitt aus der Entwicklungsphysiologie dar. Sie soll deshalb hier nur nebenbei behandelt werden, obwohl die Aufklärung einfacher Fälle der chemischen Differenzierung vielleicht am ehesten die frühen und wesentlichen Schritte der Differenzierung pflanzlicher Zellen enthüllen könnte. Ein interessanter Fall intracellulärer Differenzierung ist die Ausbildung von Galaktoplasten in Milchröhren, wobei in diesen Plastiden Kautschuk entsteht, während in den Plastiden der Parenchymzellen des gleichen Entwicklungszustandes Stärke produziert wird (Milanez, 1951).

Eine weitere enge Beziehung der Produktion sekundärer Verbindungen zur Entwicklung der Pflanzen scheint dort zu bestehen, wo solche Stoffe besonders in wachsenden bzw. sich streckenden Organen entstehen. Bei Keimlingen von *Salvia officinalis* wird ätherisches Oel nur in den jüngsten Stadien gebildet. Auch später haben bei Stengeln und Blättern nur die jungen Organe tätige Drüsen (Frey-Wyssling 1945). Aehnliches gilt für die Anthocyanbildung in Keimlingen von Rotkohl (Frey-Wyssling und Blank 1943 ; Eberhardt 1954). Bei *Asarum europaeum* entwickeln und füllen sich die Oelzellen in jungen Blättchen, in der Blütenhülle und im Rhizom während des Streckungswachstums (Paech 1952). Hier gelang es leicht durch einfache Eingriffe, die beiden Prozesse (Entfaltung der Organe und Bildung ätherischer Oele) auseinanderzunehmen. Blüten, die bei verschiedenen Temperaturen zur Entwicklung gebracht worden waren und ungefähr gleiche Grösse erreicht hatten, enthielten umso mehr Oelzellen, je höher die Temperatur lag. Hierbei zeigt sich bereits, dass die Streckung eines Organes nicht notwendig mit der Abscheidung einer bestimmten Menge ätherischen Oeles verbunden ist. Wurden Blüten, die bei niederer Temperatur zur vollen Grösse herangewachsen waren, und demnach wenig Oel enthielten, in höhere Temperatur gebracht, so differenzierten und füllten sich rasch weitere Oelzellen. Ausdifferenzierung von Oelzellen und Bildung von ätherischen Oelen sind also nicht kausal mit dem Streckungswachstum verknüpft.

Bei anderen Objekten erscheint auch unter natürlichen Bedingungen die Bildung sekundärer Verbindungen nicht mit der Streckung der Organe gekoppelt. In *Spirodela oligorrhiza* ist die Anthocyanproduktion unabhängig vom Wachstum (Thimann und Edmondson 1951). Bei *Phycomyces* wird zunächst das Mycel aufgebaut und erst dann setzt die Synthese von Carotinoiden kräftig ein (Garton *et al.* 1951 ; Goodwin und Willmer 1952). In einem gewissen Gegensatz zu den niedermolekularen Terpenen, die hauptsächlich in intensiv wachsenden, jungen Organen entstehen, wird Kautschuk am ausgiebigsten in ausgewachsenen Pflanzenteilen aufgebaut. Anders als bei den harzliefernden Bäumen, die so angezapft werden dass durch das verletzte Cambium eine Neubildung von Geweben eingeleitet wird, bleibt bei den Kautschukbäumen das Cambium verschont und die erneute Kautschukbildung tritt ohne gleichzeitiges Gewebewachstum ein. Bei *Parthenium argentatum* sammelt sich Kautchuk vor allem unter Bedingungen an, welche die Photosynthese begünstigen, aber das vegetative Wachstum zurückhalten, z. B. in kühlen Nächten und warmen Tagen (vergl. BONNER und GALSTON 1947). Bei *Hevea* wird das Austreiben der Blätter von einem Nachlassen der Kautschukproduktion begleitet. In *Taraxacum koksaghyz* steigt der Kautschukgehalt am Ende der Vegetationsperiode kurz vor Einsetzen der Ruhe besonders rasch an.

Ein tieferer Einblick in die Biogenese der sekundären Stoffe müsste erklären können, warum einerseits häufig eine Korrelation zwischen der Bildung von ätherischen Oelen, Anthocyanen, Carotinen, etc. mit dem Wachstum besteht, und warum andererseits Streckungswachstum und Produktion sekundärer Stoffe einander ausschliessen.

Der chemische Gang der Biosynthese jeder Gruppe von sekundären Stoffen lässt sich im allgemeinen in drei, allerdings nicht scharf zu trennende Asbchnitte zerlegen. Die Frage nach dem *Rohmaterial* und den *Elementarbausteinen,* aus denen ein bestimmter Typ von Pflanzenstoffen entsteht, ist ein relativ gut abgrenzbarer Bezirk, der experimentell getrennt von dem weiteren Verlauf der Synthese untersucht werden kann. Für die fetten Oele sind ohne Zweifel Kohlenhydrate der Rohstoff und Acetyl-Coenzym-A der Baustein. Für Terpene und besonders Sterine spielen Acetylverbindungen sicher auch eine wesentliche Rolle. Ob sie der einzige Baustein sind, wäre aber noch zu beweisen.

Ein weiterer Abschnitt der Untersuchung erstreckt sich auf den *Modus der Verknüpfung der Bausteine,* auf die *durchlaufenen Zwischenstufen* und die energetischen Bedingungen der Synthese einer bestimmten Gruppe von Verbindungen. Zu diesem Teil der Biosynthese sind vor allem beim Lignin einige Befunde gesammelt worden. Phenylpropanderivate wie Coniferylalkohol, Oxyzimtalkohol, Eugenol und Thymol werden sowohl im Enzymensatz als auch in der lebenden Pflanze in Lignin bzw. ein dem nativen Lignin sehr ähnliches Produkt umgewandelt (FREUDENBERG und Mitarb. 1952 ; SIEGEL 1952 ; MANSKAJA und BARDINSKAJA 1952). Der Modus der Ligninbildung besteht offenbar in einer dehydrierenden Polymerisation eines Intermediärproduktes, über dessen Entstehung man allerdings noch nichts sagen kann. Der Modus der Vereinigung von Acetylresten zu höheren Fettsäuren dürfte kein schwieriges Problem mehr darstellen. Beim Aufbau der Triterpene, besonders der Sterine dürfte Squalen eine Zwichenstufe darstellen (Vergl. RUZICKA 1953), jedoch fehlt dafür bei Pflanzen noch der experimentelle Beweis.

Ein dritter Bereich, der relativ selbständig untersucht werden kann, erstreckt sich auf die allgemeine Erscheinung, dass von jedem Typ sekundärer Stoffe ganze Serien von Verbindungen vorkommen, die sich nur in der *feineren Ausformung eines bestimmten Grundkörpers* voneinander unterscheiden. Solche nur durch Substituenten oder geringfügige Variation des typischen Bauplans, häufig durch die Zahl und Stellung der angefügten Hydroxyl-oder Methylgruppen oder durch den Oxydoreduktionszustand unterschiedene Glieder einer Serie von Pflanzenstoffen sind bei den Alkaloiden am besten bekannt. Sie bestehen auch bei den Anthocyanen und Flavonen, bei den verschiedenen niederen Terpengruppen und bei Carotinoiden und sind wohl bei anderen Gruppen sekundärer Stoffe nur noch nicht gesucht und entsprechend eingeschätzt worden. Ueber die letzten Feinheiten des Molekülbaues, die zu einer so grossen Mannigfaltigkeit der sekundären Verbindungen führen, sind durch Untersuchung der Vererbbarkeit bei Anthocyanen und Carotinoiden einige Einblicke in chemische Zusammenhänge gefunden worden (SCOTT-MONCRIEFF 1939 ; ONSLOW 1932 ; PORTER und LINCOLN 1950 ; LINCOLN und PORTER 1950).

Die genaue Kenntnis des allgemeinen Kohlenhydratabbaues und anderer primärer Umsetzungen (z. B. die Synthese polymerer Naturstoffe aus phosphorylierten Bausteinen) legt es nahe, danach zu suchen, ob und in welchem Umfange die sekundären Verbindungen sich von den normalen Intermediärverbindungen des durch eine grosse Einheitlichkeit in allen Organismen ausgezeichneten allgemeinen glykolytischen Umsatzes, aus dem Tricarbonsäurekreislauf und dem Auf-bzw. Abbau der Aminosäuren, also aus primären Umsetzungen, ableiten. Die andere unwahrscheinlichere Alternative wäre, dass spezielle Umwandlungen bereits am Rohmaterial, selbst, also an den Kohlenhydraten, ansetzten und unabhängig vom Grundstoffwechsel zur Entstehung der sekundären Substanzen führten.

Dass *Kohlenhydrate das Rohmaterial* für sehr viele N-freie sekundäre Verbindungen darstellen, ist heute nicht mehr zu bezweifeln. Das ist an so vielen Beispielen gezeigt worden, dass hier nur zwei neuere Befunde für Carotinoide erwähnt werden sollen. Wenn *Phycomyces blakesleeanus* nur 1 % Glucose im Nährmedium bekommt, wächst das Mycel zwar noch sehr gut, aber die Carotinbildung bleibt aus. Erst mit 3 % Glucose werden Carotinoide produziert (GOODWIN und LIJINSKY 1951). In etiolierten Weizenkeimlingen nimmt in den ersten 8 Tagen die Carotinmenge rasch zu. Obwohl die Keimlinge dann noch weiter wachsen, bleibt der Carotingehalt pro Kemling zurück. Bei Zufütterung von Glucose bilden die Keimlinge auch nach dem 8. Tage noch Carotinoide (BEEKMANN 1953).

Die reichlichere Ausstattung mit allerlei sekundären Verbindungen der tropischen und subtropischen Pflanzen gegenüber den Gewächsen gemässigter und kalter Zonen dürfte ihre tiefere Ursache in einer besseren Versorgung mit Assimilaten haben. Aus dem gleichen Grunde sind Schattenpflanzen und Submerse im Gehalt an sekundären Stoffen unterlegen. Heterotrophe Pflanzen sind im allgemeinen ebenfalls arm daran. Pilze bilden die für sie charakteristischen Stoffwechselendprodukte oft nur bei der luxuriösen Versorgung mit Nährstoffen, die sie im Laboratorium geniessen (vergl. FOSTER 1949).

Genauere Untersuchungen erweisen jedoch, dass nicht Kohlenhydratanhäufung allein die Produktion von sekundären Stoffen in Gang bringt oder fördert, sondern dass dazu ein erhöhter *Umsatz des plastischen Materials* nötig ist. Wir haben das an verschiedenen Objekten nachgewiesen. In Rotkohlkeimlingen wird Anthocyan dann besonders intensiv gebildet, wenn das Reservefett mobilisiert wird. Nach Zuckergabe zu Blättern von *Saxifraga crassifolia* steigt die Atmung (O_2-Aufnahme und CO_2-Abgabe) stark an und es wird reichlich Anthocyan gebildet, wenn die Blätter in mässigem Licht aufbewahrt werden. Im Dunkeln nehmen die Blätter zwar auch Zucker auf, aber der Zucker wird nicht umgesetzt, wie aus dem viel geringeren Gaswechsel hervorgeht, und es wird dementsprechend kein Anthocyan gebildet (PAECH und EBERHARDT 1952). Hierher gehört wohl auch die Anthocyanbildung bei der herbstlichen Laubfärbung, die mit erhöhtem Gasumsatz verbunden ist, die Ausfärbung der Früchte während der Atmungssteigerung im « climacteric rise » und ähnliche Erscheinungen.

In allen diesen Fällen sind aber immer noch Bedenken angebracht, ob der Zusammenhang zwischen erhöhtem Gasumsatz und gesteigerter Bildung von Pigmenten durch den Anfall von Intermediärprodukten der Glykolyse bewirkt wird (vergl. dazu EDDY und MAPSON 1951 ; GEISSMAN und HINREINER 1952 ; THIMANN *et al.* 1951). Noch andere experimentelle Wege müssen zur Entscheidung dieser Frage begangen werden. Die Verwendung von markierten Verbindungen, die der intakten Pflanze zugegeben werden, scheint allen anderen überlegen zu sein. Doch gerade am Beispiel der Carotine ist gezeigt worden, wie leicht Isotopenversuche auf Irrwege führen können. Es darf nicht ohne weiteres auf eine direckte Umwandlung der markierten Ausgangsverbindung zum Endprodukt geschlossen werden, auch wenn dieses einen grossen Teil der Aktivität aufweist. Besonders gewagt ist dieser Schluss, wenn es sich um eine lange Versuchsdauer und um recht allgemeine Intermediärverbindungen handelt. Bei *Phycomyces blakesleeanus* hatten GROB *et al.* (1951) den Einbau eines hoben Prozentsatzes von Acetat, das sowohl in der Methyl-als auch in der Carboxylgruppe markiert war, festgestellt. Da auch andere, inzwischen allerdings wegen methodischer Fehler entwertete Tatsachen (SCHOPFER und GROB 1950) Acetat als direkten Baustein der Carotinoide erscheinen liessen, war man eine Zeitlang der Meinung, die Carotinoide würden direkt und allein aus Acetat zusammengebaut. GOODWIN und LIJINSKY (1951) und GOODWIN (1953) haben das widerlegt. Der entscheidende Baustein für die Kautschuksynthese scheint nach Versuchen mit *Parthenium argentatum* ebenfalls Acetat zu sein (BONNER und ARREGUIN 1949 ; ARREGUIN und BONNER 1950). Im Prinzip wurde dabei in ähnlicher Weise wie bei der Carotinoidbildung die Bedeutung von Acetat ermittelt. Ob hier auch die gleichen Bedenken angebracht sind ?

Eine weitere Vorsichtsmassnahme bei der Fütterung von vermuteten Zwischenverbindungen hat sich ebenfalls bei der Carotinbildung in *Phycomyces* als besonders nötig erwiesen. Bestimmte Aminosäure, z. B. Leucin und Valin, steigern die Carotinsynthese nur, wenn im Nährmedium wenig Glucose vorhanden ist. Eine zugegebene Verbindung, die als Baustein geeignet ist, kann nur dann eine Mehrproduktion bewirken, wenn die zelleigenen Baustoffe so weit erschöpft sind, dass die Kapazität des Stoffwechsels (bedingt durch Menge der Enzyme u. a.) nicht schon voll ausgenutzt ist. In einem gesättigten Stoffwechsel können zusätzliche Baustoffe nicht mehr verwendet werden, auch wenn sie den genuinen Zwischenverbindungen entsprechen.

Als weiteres Mittel, Bausteine und Zwischenprodukte sekundärer Pflanzenstoffe und den Ort ihrer Abzweigung aus dem allgemeinen Stoffwechsel ausfindig zu machen, kommt *das Abfangen früher Vorstufen* in Betracht. *Lentinus lepideus,* ein holzzerstörender Pilz, bildet auf verschiedenen Zuckern (Glucose, Xylose) aber auch auf Aethylalkohol eine aromatische Verbindung (p-Methoxyzimtsäure-methylester). Dabei kann Acetaldehyd als Zwischenprodukt abgefangen werden (NORD und VITUCCI 1947), was dafür spricht, dass wenigstens in diesem Falle die Synthese aromatischer Verbindungen, und zwar einer für den pflanzlichen Stoffwechsel so charakteristischen C_6-C_3-Verbindungen, über die normalen glykolytischen Zwischenprodukte, seien es nun C_2-Körper oder sei es Brenztraubensäure, verläuft.

Wenn auch nicht immer so leicht definierte Zwischenprodukte abgefangen werden können, so gelingt es doch manchmal zu zeigen, dass primärer Stoffwechsel, z.B. die Aminosäuresynthese, und die Bildung sekundärer Stoffe aus der gleichen Quelle gespeist werden. Bei etiolierten Weizenkeimlingen, die bis zum 8. Tage des Wachstums Carotinoide ansammeln, bewirkt Zugabe von verwertbaren Stickstoffverbindungen, z.B. 0,1 % ige Harnstofflösung, eine erhöhte Eiweissynthese, gleichzeitig wird die Carotinbildung zurückgedrängt (BEEKMANN 1953). Wenn ausgebildete Myceldecken von *Phycomyces* auf ein N-freies glucosehaltiges Medium übertragen werden, bilden sie relativ mehr Carotinoide, als wenn Stickstoff anwesend ist (GOODWIN und WILLMER 1952). Bei höheren und bei niederen Pflanzen setzt also konkurrierend die Synthese von Aminosäuren (= Eiweissbausteine) und Carotinoiden an der gleichen Quelle an.

Eine andere Art von Abfangtyp, den die Natur selbst darbietet, und der ein Zwischenprodukt der Terpensynthese festhält, dürfte in solchen Verbindungen gegeben sein, in denen das Prenol oder andere Verbindungen mit den charakteristischen gegabelten C_5-Element enthalten sind. So liegt im Foeniculin das Prenol mit einer Phenylverbindung des charakteristischen C_6-C_3-Typs veräthert vor ; im Galegin ist Prenol mit dem Guanidinrest kondensiert, und im Lavandulol sind 2 Prenolmoleküle durch eine ungewöhnliche Kondensation so miteinander verknüpft, dass die Struktur des C_5-Bausteines, der für sich selbst nicht beständig ist, deutlich erkennbar bleibt. Andere Verbindungen, die das frei in der Pflanze nicht beständige Prenol fixiert haben, bestärken die Ueberzeugung, dass Prenol oder eine damit sehr nahe verwandte Verbindung tatsächlich die Rolle einer frühen Zwischenstufe bei der Terpensynthese spielt.

Schliesslich bieten sich *Eingriffe in den glykolytischen Umsatz* an, aus deren Folgen man Schlüsse über die Ableitung der sekundären Stoffe aus dem allgemeinen Umsatz ziehen und im günstigsten Falle vielleicht sogar den Ort der Abzweigung durch Hemmung der Spaltung an bestimmter Stelle angeben könnte. Wir haben mit einer Reihe der bekannten Hemmstoffe für Enzyme des glykolytischen und oxydativen Umsatzes in solche höhere Pflanzen eingegriffen, welche Anthocyane oder Carotine bilden (PAECH und EBERHARDT 1952 ; EBERHARDT 1954 ; BEEKMANN 1953, 1954). Die Schwierigkeiten dabei liegen nicht nur in der Anwendung der Enzymgifte auf intakte höhere Pflanzen, sondern vor allem darin dass die Wirkung der bisher gebräuchlichen Enzymhemmstoffe nicht eindeutig ist. Aus den Versuchen mit Enzymhemmstoffen lässt sich aber jetzt schon soviel entnehmen, dass Phosphorylierungen sowohl für die Anthocyan-als auch für die Carotinsynthese wesentlich sind, weil 2,4-Dinitrophenol hemmt. Bei Weizenkeimlingen wurde ausserdem wahrscheinlich gemacht, dass die Carotinbausteine nicht mit dem Acetat allein gegeben sind, sondern dass Glieder des Citronensäurecyclus zwischen Essigsäure und α-Ketoglutarsäure noch von Bedeutung sein müssen. Da Jodessigsäure die Anthocyansynthese hemmt, müsste noch der Umsatz 3-Phosphoglycerinaldehyd = Phosphoglycerinsäure zur Anlieferung der Bausteine erforderlich sein.

Damit mögen die Andeutungen, wie eine einheitliche Untersuchung und Betrachtung der Biogenese vieler sekundärer Stoffe angegangen werden kann, genug sein.

Mit einer gewissen Vorsicht dürfen heute vielleicht schon einige allgemeine Regeln, die die Biogenese sekundärer Stoffe beherrschen, abgeleitet werden.

1) Die Voraussetzung für die Synthese sekundärer Stoffe ist eine reichliche, über die Bedürfnisse des Wachstums hinausgehende Versorgung mit mobilisierbaren Kohlenhydraten oder anderen plastischen C-Verbindungen.

2) Nur wenn dieses Rohmaterial durch den allgemeinen Umsatz mobilisiert wird, kommt die Synthese wirklich in Gang.

3) Die Synthese wird aus einem Reservoir allgemeiner Intermediärverbindungen gespeist, aus denen auch die Synthese von Aminosäuren und anderen lebenswichtigen Zellbestandteilen ihre Bausteine entnimmt.

4) Ueber die Beziehungen zwischen Streckungswachstum und Synthese sekundärer Verbindungen (nach Beobachtungen über ätherische Oele, Carotine, Anthocyane, Kautschuk) lässt sich sagen, dass die allgemeinen Bausteine an die Orte der Zellstreckung entweder in so grosser Menge geleitet werden (Vorherrschen des spaltenden Umsatzes an den Orten des Zellwachstums GODDARD 1948), dass das durch andere Faktoren begrenzte Wachstum nur einen Teil davon verbraucht, so dass die im Zuge der Zell und Gewebsdifferenzierung sich entwickelnden Fähigkeiten zu speziellen

Synthesen noch genügend Baumaterial finden und sekundäre Stoffe gleichzeitig mit der Zellstreckung anfallen (simultane Bildung), oder aber das durch keine anderen Faktoren begrenzte Streckungswachstum verbraucht die zugeleiteten Bausteine restlos, so dass erst nach Abklingen der Streckung die Produktion sekundärer Stoffe genügend Material zur Verfügung hat (sukcedane Bildung). Besonders in diesem Falle wird durch Fütterung von geeigneten Bausteinen oder durch Hemmung des Wachstums, etwa bei N-Mangel, eine gesteigerte Bildung erzielt.

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OLEORESINOGENESE ET GEMMAGE

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Pour « gemmer » un Pin suivant la méthode classique, on pratique une entaille dans le tronc de l'arbre et on recueille l'oléorésine qui s'écoule de la blessure ; on renouvelle cette dernière périodiquement ; cette blessure intéresse l'écorce, le liber, le cambium et une partie du bois. Mais on obtient une quantité plus importante de gemme (30 à 50 %) par la technique du « gemmage activé ». Dans ce cas, l'opérateur enlève seulement les tissus jusqu'au cambium et pulvérise sur la plaie une solution d'acide sulfurique à 60 % ; une nouvelle blessure de 8 cm² environ est pratiquée tous les quinze jours au-dessus de la précédente.

L'oléorésinogenèse a fait l'objet de divers travaux. **Devaux** et **Bargues** ont montré notamment que toutes les cellules du Pin maritime sont susceptibles de donner de l'oléorésine. Ils ont constaté, en outre, pour les aiguilles, que cette dernière disparaît à peu près complètement pendant la journée et se reforme pendant la nuit, aux dépens de l'amidon, semble-t-il, car c'est l'inverse qui se produit pour ce glucide : il apparaît pendant la journée et disparaît, en partie au moins, pendant la nuit. La genèse de l'oléorésine paraît ainsi plus ou moins directement liée à la glucogenèse.

A la suite de ces travaux, nous avons entrepris une étude comparée de la teneur des aiguilles en glucides et oléorésine dans le cas des arbres gemmés (méthodes Hugues et gemmage activé) et témoins (non gemmés). L'un de nous (1) ayant constaté que l'activation du gemmage par l'acide ne paraît pas diminuer la vitalité de l'arbre (la vitesse de la croissance est la même dans les deux cas et l'observation cytologique indique que l'application d'acide sulfurique ne provoque qu'une altération locale), l'accroissement de la coulée de gemme devrait donc provenir d'une stimulation générale du métabolisme. Nous avons tout d'abord recherché si une telle stimulation peut être décelée. Nous résumons dans la précédente note nos premiers résultats relatifs aux glucides (teneur en glucides solubles réducteurs ou non réducteurs et glucides insolubles hydrolysés au bain-marie en présence de SO_4H_2) contenus dans les aiguilles des pins traités par les deux méthodes.

Les prélèvements soumis à l'analyse ont été effectués dans le courant de la matinée, d'une part pendant la période de gemmage et de pleine activité métabolique (mai 1953), d'autre part pendant la période hivernale : pas de gemmage, activité métabolique réduite (janvier 1954). Des extrémités de rameaux latéraux ont été ainsi coupées sur 10 arbres de 38 ans, dans la partie médiane de leur masse feuillée (vers 16 m. de hauteur). Cinq arbres étaient gemmés par la méthode française, les cinq autres par la technique de gemmage activé. Ces arbres se trouvaient dans deux parcelles aussi semblables que possible, tant en ce qui concerne la constitution du sol que la vigueur des sujets.

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(1) R. **David**. *Bois et Résineux*, n^{os} 1.573 à 1.576. 30 nov., 10 et 20 déc. 1951. 10 janv. 1952. *C. R. Acad. Sc.*, 1953, t. 236, p. 2.335.

TABLEAU I.

	CARE HUGUES	CARE ACTIVEE
Prélèvement du 13 Mai I953		
longueur moyenne d'une aiguille, exprimée en centimètres.	12,5	12
poids frais moyen d'une aiguille, exprimé en grammes.	0,52	0,56
poids sec moyen d'une aiguille, exprimé en grammes.	0,24	0,26
teneur moyenne en eau d'une aiguille.	48,6 %	46,9 %
Prélèvement du 22 Janvier I954		
longueur moyenne	12,1	12,8
poids frais	0,428	0,515
poids sec	0,186	0,210
teneur en eau	56,5 %	59,2 %

Les caractéristiques des arbres étudiés étaient les suivantes. En ce qui concerne le gemmage, les uns avaient été traités pendant huit ans par la méthode française, les autres d'abord pendant trois ans par le même procédé, puis pendant cinq années par le « barck chipping » activé à l'acide. Dans le tableau I sont mentionnées les valeurs moyennes des caractéristiques des aiguilles étudiées.

L'examen des résultats indiqués dans ce tableau I montre que les aiguilles des arbres traités par la méthode de gemmage activé ont des poids et des teneurs en eau sensiblement supérieurs à ceux des aiguilles des arbres traités par la méthode Hugues.

Le dosage des glucides a été effectué par la méthode classique après fixation du matériel à l'alcool bouillant. Les résultats obtenus sont mentionnés dans le tableau II.

TABLEAU II.

	CARE HUGUES		CARE ACTIVEE	
	mg. par feuille	mg. par g. de matière sèche	mg. par feuille	mg. par g. de matière sèche
PRELEVEMENT DU 13 MAI 1953				
glucides solubles réducteurs, exprimés en glucose	2,4	9,4	3,5	13,6
glucides solubles non réducteurs, exprimés en saccharose	3,9	13,7	7	26,6
glucides insolubles, exprimés en amidon	79	315	81,7	316
PRELEVEMENT DU 22 JANVIER 1954				
glucides solubles réducteurs	3,5	18,8	5,2	25,4
glucides solubles non réducteurs	5,5	29,5	7,2	34,3
glucides insolubles	34,3	184	38,5	184

Ainsi, on remarque que les aiguilles contiennent davantage de glucides solubles en hiver qu'au printemps ; c'est le phénomène inverse que l'on observe pour les glucides insolubles. Mais quelle que soit l'époque du prélèvement, la quantité de glucides solubles, réducteurs ou non réducteurs, est toujours nettement supérieure dans le cas du gemmage activé ; la teneur en glucides insolubles est sensiblement la même dans les deux cas.

En résumé, d'après les dosages qui ont été effectués jusqu'à maintenant, il semble bien que l'accroissement de la sécrétion de l'oléorésine, sous l'influence du double traumatisme mécanique et chimique (gemmage activé), soit concomitante d'une stimulation de la glucogenèse. Certes, il est nécessaire de poursuivre cette étude physiologique pendant un temps assez long, et surtout d'attendre les résultats des dosages (actuellement en cours) des acides résiniques des aiguilles pour conclure d'une manière ferme ; mais, d'après ceux que nous possédons déjà, il semble qu'il soit possible que l'action de l'acide se traduise bien par une stimulation générale de l'anabolisme de l'arbre.

4. *NUTRITION ET CARENCES MINERALES*

PREMIER RAPPORT

MODERN METHODS OF DETERMINING THE MINERAL NUTRIENT REQUIREMENT OF PLANTS WITH SPECIAL REFERENCE TO THE MICRONUTRIENT ELEMENTS

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In reviewing modern methods used in studying mineral nutrition of plants it is necessary to recall that many ideas widely accepted often only recently were introduced early in the history of the subject. Credit is due particularly to Woodward, Wiegman and Polstorf, Salm-Horstmar, Sachs, Knop, Nobbe, Raulin, Javillier, and Bertrand and Javillier, whose pioneer investigations (1) were responsible for introducing early ideas and evidence on such points as water purity, seed reserves, sand purification, water culture, molar nutrient ratios, constant solution renewal, aeration, effects of containers, nutrient purification and evidence of essentiality of manganese and zinc.

Thus modern methods do not comprise much that is new in principle. It is in the recognition of the importance of the principles involved and in their efficient and specific application to the solution of particular problems that the modern methods have developed. This progress is closely related to progress in the recognition of the known essential micronutrients and in the continued search for evidence of others, and attention will be devoted mainly to this field here. An unbiassed approach combined with the imagination and initiative to grasp the significance of unexplained observations has often been an important factor in the subsequent elucidation of micronutrient status by controlled methods. At least two major contributions regarding molybdenum and vanadium have arisen thus (2, 3).

Without distinction between those providing the first clue or conclusive evidence, it is appropriate here to give especial credit to the following for their contributions regarding the elements named (1). Boron : Aghulon, Mazé, Warington ; Manganese : Salm-Horstmar, Raulin, Bertrand and Javillier, McHargue, Samuel and Piper ; Zinc : Javillier, Mazé, Steinberg ; Copper : Sommer, Lipman and MacKinney, Piper ; Molybdenum : BORTELS, STEINBERG, ARNON and STOUT, PIPER ; Vanadium : BORTELS, BERTRAND, ARNON and WESSEL (3) ; Cobalt : MASKELL, EVANS and MURRAY (4) ; STOUT, BROYER and JOHNSON (5).

Results obtained in tests with gallium, silicon, chlorine, selenium and aluminium are not so readily interpreted and unexplained effects have been recorded in some instances.

Factors affecting incidence of micronutrient deficiencies.

Modern methods are best appreciated from a survey of sources of interference and their control (1). They comprise the containers, solid media, if used, water, nutrient reagents, dust, volatile materials and the initial seed or parent material.

Containers : These may cause contamination from adsorbed or soluble constituents. The first may be removed usually by acid or alkaline treatment with steaming or autoclaving or by contact with a chelating substance. Soluble contamination is more serious. Interference is decreased by prolonged use. Mechanical defects such as hair cracks seriously interfere with cleaning. Borosilicate glass is widely used and is usually superior to glazed materials where boron and zinc are both present. Silica and plastic materials, especially polythene, are some of the most inert. Unglazed clay pots painted with a pure bitumen solution are inexpensive and satisfactory for boron, iron, manganese and zinc deficiencies but not for copper or molybdenum. Rubber may be a serious source of zinc or of barium and should always be extracted by hot 1:2 hydrochloric acid. Plastic tubing is probably preferable.

Solid media : Silica sand is most commonly used and may provide many impurities adequate for the growth of the plants. Plastic granules and pyrex glass fragments with glass wool have also been used. A method is now available for treatment of sand on a large scale with acid or alkali and steam which removes most of the micronutrient impurities (1). The acid soluble molybdenum content may be 0.01 p.p.m. initially and this is decreased to about 0.00001-2 p.p.m. available molybdenum. Severe deficiencies of six of the known micronutrient elements can readily be produced in this medium.

Water supplies : The large quantities of water required by culture plants may introduce significant amounts of nutrients. Their nature varies with the origin, treatment and storage of the water. Limits to the purity of distilled water are set by problems of spray or creep, the nature of the condensing surface and frequency of cleaning. Best results are probably obtained from pyrex glass or silica stills. A pyrex condenser united to a metal boiling chamber through a long column and splash trap may yield water low in heavy metals (6), but in some designs this method is unsatisfactory.

Ion exchange resins provide satisfactory water for culture experiments. The water compares well with glass distilled water with respect to the micronutrients and is used for production of iron, manganese, boron, copper, zinc and molybdenum deficiencies on a large scale at Long Ashton (1).

The use of mixed-bed ion exchange resins should allow an approach to the asymptotic limits of the exchange reactions and the composition of the resins and their containers may become an important limiting factor in final purity. The presence of un-ionised compounds or highly stable complexes, e. g. trivalent cobalt porphyrins will also limit the final purity attainable. A delicate test for heavy metals in water uses dithizone (7). Molybdenum is detected by assay with *Aspergillus niger* (1).

Nutrient reagents : Many reagent grade or « pure » compounds used to provide the major elements may be serious sources of contamination by several micro elements and their specified grade is no criterion of purity in this respect. Iron compounds and micronutrient salts are supplied in smaller amounts but their compounds are often heavily contaminated and may contribute significant interference especially in work on previously unrecognised essential elements, e. g. vanadium where differences in iron supply gave the clue to the discovery (3). Purified iron compounds are therefore always to be recommended.

The methods used to purify nutrient reagents merit description. Simple recrystallisation may be ineffective owing to isomorphism or mixed crystal formation but is useful for removal of boron (1). Addition of a chelating agent during recrystallisation has recently been adopted (8). Adsorption by calcium phosphate in alkaline solution is one of the oldest routine methods introduced by Steinberg (9) and with various modifications (7, 1) is a reliable procedure for removal of iron, manganese, copper and zinc, and probably cobalt and other elements. It is not effective for the removal of molybdenum and its relation to initial results with molybdenum (2) were probably fortuitous. The removal of molybdenum may be effected by co-precipitation with iron or aluminium and 8-hydroxyquinoline (9 a) (1) or by co-precipitation with copper sulphide lead sulphide (10) (1) or mixed sulphides (1) (16). Sulphide co-precipitation apparently removes vanadium (3) and with suitable choice of collecting metal and of pH is probably highly effective in removing several elements including cobalt. The method can be applied to iron compounds, citrates, amino acids, sugars and alkaline solutions. Extraction by immiscible solvents is frequently used together with a chelating agent (1), e. g. diphenyl thiocarbazone for copper and zinc, cupferron for vanadium, 8-hydroxyquinoline for zinc. The ether-concentrated hydrochloric acid system devised by Rothe has been applied in the preparation of iron compounds free from manganese, copper, zinc, and vanadium. It will also separate ferric iron from cobalt and cadmium but not from molybdenum or gallium. By starting with ferrous iron their further separation can be achieved prior to oxidation and further extraction (1). Adsorption on charcoal was formerly much used (1) *loc. cit.*, but is not wholly advisable owing to the possible simultaneous introduction of numerous ash constituents. It may have special applications, e. g. in removal of rare earth metals as organic complexes. Ion exchange resins have been used to purifiy non-ionised solutes e. g. sugars with notable success.

In all methods it is important to take into account possible residual effects due to traces of chelating agent, solvent or sulphide remaining in the final product which may cause unexpected effects. Careful control tests are then of special importance in searching for other essential elements. It is evident that a given method may be effective only for specific elements and that distinction between success or failure may rest on small points of detail.

Dust and atmospheric contamination : Dust may be a critical factor in work on micronutrient deficiencies, and zinc contamination from this source may be adequate for plant growth. Many micronutrients are present in dust. Covers are easily provided for water cultures but are not easily adapted for sand culture owing to the relationship of the plant development to its growth in the sand. Dust and volatile substances, e. g. sulphur, mercury or iodine are not readily excluded and may be absorbed through foliage. Air conditioned cabinets may be required when such possibilities arise.

Seed reserves : It is probably axiomatic that the seed or other parent material must have contained all elements essential for the species. For many practical purposes this is not important. Seed or tuber reserves, however, may be determining factors in the response to molybdenum (11) (12), copper (12) or other elements and are obviously to be accounted in attempts to show the essential need of other elements. Use of seed impoverished by raising it under deficiency conditions for one or more generations has been reported (1). It is possible that in early work the results attributed to chlorine or silicon might have been due to molybdenum or copper for example contained as impurities in the chloride used. Some elements, e. g. zinc, do not appear to decrease much in the seed and failure to produce seed is the only response obtained.

Use of sand or water cultures : Water cultures have the advantages of eliminating the contamination from a solid medium and of being easily covered. They also have three technical limitations. (a) Chlorosis arises frequently from iron deficiency and plants differ widely in their susceptibility. Iron combined with ethylene diaminetetra acetic acid has recently been used to overcome this problem (13) and has been found satisfactory in molybdenum deficient cultures of *Chlorella* (14). (b) Aeration is often a limiting factor. Forced aeration is usually required. It may need regulation for some plants and may introduce oil spray or dust into the cultures unless carerully filtered air is used. (c) Some plants, e. g. cauliflower or apple trees do not grow satisfactorally in normal water culture as compared with sand cultures (1). Special methods have been devised to spray fine droplets of solution on to tree roots and normal growth occurs (15). Much extra equipment is needed and may complicate work with micronutrient deficiencies. Sand cultures have been especially useful in showing the essentiality of molybdenum for cauliflower grown with ammonium sulphate when the addition of solid calcium carbonate was necessary (1).

Limits and validity of methods.

Three criteria have been suggested by ARNON to test the evidence of essentiality of an element. These are (a) that omission interferes with growth or reproduction, (b) that the element may not be substituted by another, (c) that the effect is direct and not the result of some antagonism. A negative response does not indicate non-essentiality, only that if essential, the limits are less than those set by the experiment.

It is especially important to apply the third criterion in assessing evidence of essentiality. Some heavy metals may induce chlorosis indicative of iron deficiency, a cobalt-manganese antagonism is likely and a similar antagonism may occur between copper and aluminium. These examples show how results might be misleading.

Application of the criteria may be difficult in special instances where elements are mutually replaceable, e. g. molybdenum and vanadium or calcium and strontium as neither one of a pair would appear to be essential in the presence of the other but one or other must be present. Provision of a specialised medium may so lower or eliminate the requirement of an element as to limit the application of the criteria to special circumstances.

Quantitative tests of Methods.

It is important to attempt some estimate of the probable concentration below which the element must be tested in the hope of a response. Chemical or spectographic tests are often insufficiently sensitive even if carried out on the plant material which serves as a concentrating mechanism. A quantitative biological test is the only measure of the total available concentration but can only be applied if there is an organism known to require the element. The selection of a suitable test organism may be important. A large vegetative growth to seed or spore ratio, independance of photoperiod, autotrophic nutrition, and easy quantitative estimation of yield are desirable features. Organisms such as *A. niger* requiring organic carbon as the only known organic supplement are also useful. It is probably correct to assume that higher plants are truly autotrophic in culture experiments and do not require organic growth factors but the problem may have exceptional aspects of controversial interest (1).

Aspergillus niger is an outstanding example of sensitivity and will detect 10^{-4} µg. molybdenum (1), (16). It would now appear that *Scenedesmus* (3) is able to detect vanadium at low concentrations. Cobalt might be detected indirectly by a double culture method using the *Lactobacillus Leichmanii* or *Euglena gracilis* B_{12} assays to detect B_{12} in an organism such as *A. niger* (16) able to synthesise this compound from glucose, nitrate and cobalt but for which it appeared not to be essential. This method requires the assumption that the calculated amount of cobalt found is not much less than the total present. The identification of stable cobaltoproteins in *Neurospora* places this assumption in some doubt.

In the absence of biological assay, radio-active isotopes may be used to estimate the efficiency of nutrient purification methods at submicrogram levels. They are especially useful in rapid surveys of tentative methods and are independent of other sources of contamination, but conversely cannot assess them. Complete information on the whole method can only be derived from the combination of both tests. Tracer methods also permit estimates of the absorption of micro elements from submicrogram concentrations in the medium.

Conclusions

The following points may be emphasised from this survey. The nutrient medium must provide from *known* sources *all known* or *probably essential* elements in expected physiological concentrations to avoid accidental deficiency or excess conditions producing erroneous results. Any investigation on the essentiality of micro elements should apply all the principles and relevant methods discussed to avoid unnecessary possibilities of contamination.

Use of the methods described here has led to several major advances in the study of plant micronutrient requirements. Thus we are able consistently to demonstrate under reproducible conditions the essential nature of at least 6, and probably 8, micronutrient elements required by many representatives of higher plants or micro-organisms. The effects of some of these elements can now be estimated quantitatively at concentrations of the order of a millimicrogram per litre. Precise information on the requirements and on growth responses to controlled increments at millimicrogram levels are obtainable not only for micro organisms in limited cultures but for higher plants grown in large scale investigations (1) (17). It is thus possible to produce the type of biological material closely standardised in the required mineral status which is fundamentally necessary for studying the role of mineral elements in growth and metabolism, and especially useful in revealing differential effects associated with an element such as molybdenum for which the optimal requirements or competitive demands of possible multiple roles may differ in the plant (17).

Finally a cautious speculation may be offered regarding the evolutionary relationships in the role of essential micronutrients which may be of significance in our investigations. It may be postulated that where an element has fulfilled a multiple role, (as many do) in systems of possibly low specificity with respect to the activating element, the possibility of activation by alternative but related elements arises eg Ca or Sr and Mo or V. With increasing evolutionary specificity and differentiation the alternative elements may become specific for the different functions they once shared and both then become essential, a possible example being Mo and V in *Scenedesmus* (3). On the other hand such increased specificity may result in the exclusion of one or other from both roles. Differentiation between organisms may equally result in one element being essential for one organism and the other being essential for another in parallel roles, but no longer mutually replaceable. Thus we may expect that some elements may be essential only for certain organisms evsn though they are alternatives or useless in others. The status of the « beneficial » elements is also involved. Of two elements, one alternative activating element may be the more efficient of the two *in vivo* for a particular system but the other may have other roles in which it cannot be replaced or is the only one to fulfil the multiple roles at relatively optimal efficiencies. The first element is not therefore necessarily essential but may be physiologically preferable or comparable for a specific purpose, and the distinction may be of economic importance. For example, CROWTHER has shown that sodium may be preferable to potassium for sugar production in sugar beet although sodium can only be classed as a beneficial element.

We should therefore require extensive evidence of essentiality in relation to phylogeny before accepting the conclusions that an element may be of wide or universal importance. Conversely, we must be prepared to give complete acceptance to even a single instance (3) provided the work is acceptable by modern standards and the three criteria appear to be satisfied.

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EFFECT OF POTASSIUM CONCENTRATION ON THE TIME COURSE AND RATE OF K^{42} UPTAKE BY SUNFLOWERS AS OBSERVED WITH A LOCALIZING SCINTILLATION COUNT-RATE METER

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A negative linear proportionality is suggested to exist between the fresh weight of a plant and the osmotic tension of its nutrient solution. There is also evidence that the rate of ion uptake by various plants can be independent of the ionic concentration. It was the purpose of this series of experiments to study the time course of K^{42} uptake of young sunflower plants in the light of these considerations. Sunflowers (*Helianthus annuus*) were grown in vermiculite with number 2 Hoagland's solution of an osmotic pressure of 0.77 atmospheres. When the fourth pair of leaves was sufficiently large a series of plants was transferred to a concentration series of $KHCO_3$ with osmotic pressures from 0.3 to 28.2 atmospheres. Each plant was also provided with 43.4 mg. of $K^{42}HCO_3$. To record continuously the accumulation of K^{42} in one leaf of the fourth pair the detector head of a recording scintillation countrate meter was placed 1.6 mm above the midrib of the leaf about 2 cm from the petiole. Calibrations of the instrument indicated an approximate detection area of 1.3 cm². It was found that the relation between the period of K^{42} uptake and its appearance in the leaf was composed of three stages : 1) an initial and rapid accumulation ; 2) a period of apparent saturation ; 3) a final period of accumulation. The initial phase proceeded with a rate of accumulation which varied inversely with the salt concentration of the nutrient medium. It lasted for not more than 200 minutes. The midpoint of the subsequent phase of saturation with K^{42} occurred about 530 minutes after the addition of the isotope. The final phase of accumulation began abruptly after about 1,000 minutes. It coincided with the appearance of the typical symptoms of leaf damage due to excessive concentrations of salt and of potassium. (This abstract is based on the *Second Quarterly Report*, Biol. Div., O.R.N.L., 1952).

UEBER EINIGE METHODEN ZUR BERECHNUNG DER PROTOPLASMA-PERMEABILITAT (SUR QUELQUES METHODES POUR CALCULER LA PERMEABILITE PROTOPLASMIQUE)

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La perméabilité passive est définie comme la pénétration dans la vacuole d'une substance par la pellicule protoplasmique. La substance est dissoute dans l'eau comme milieu extérieur et son transport se fait sans conversion d'énergie. La quantité transportée se laisse déterminer par différentes méthodes. Plusieurs d'entre elles, valables pour les anélectrolytes, sont considérées ici.

Pour calculer la résistance protoplasmique contre cette pénétration, la loi de Fick est confirmée comme base de calcul et est appliquée aux cellules cylindriques dans des solutions hypertoniques pour lesquelles la quantité introduite est facile à calculer si l'équilibre osmotique s'est installé. Les phases d'une telle expérience sont indiquées et les conditions pour lesquelles on peut déduire du changement du volume vacuolaire le facteur pour la résistance protoplasmique sont discutées. Quelques méthodes différentes pour calculer ce facteur sont citées, dont une tient compte des variations permanentes de la surface du protoplasme et de la concentration de la substance transportée au cours de l'expérience :

$$K = \frac{b}{4} \cdot \frac{L_2 - L_I - \frac{b}{3} \cdot \ln \frac{L_2}{L_I}}{\left(L_0 - \frac{b}{3}\right)\left(t_2 - t_I\right)} \qquad \mathrm{I}$$

(K = facteur de perméabilité [inverse de la résistance] du protoplasme en cm/sec ; b = largeur de la cellule cylindrique en cm ; L_1 et L_2 longueur de la vacuole d'une extrémité à l'autre, en cm, pour les temps $_1$ et t_2, en sec ; L_0 longueur extrapolée pour le commencement de l'expérience).

Selon cette formule, la dilatation de la vacuole est proportionnelle au temps et sa vitesse est plus faible si la solution est plus concentrée. Ce comportement est confirmé par des expériences. Les relations entre la quantité totale pénétrant dans la vacuole, sa valeur pour l'unité de la surface vacuolaire et sa concentration au cours de l'expérience sont discutées.

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ACTION DES TRAUMATISMES SUR LA TRANSPIRATION VEGETALE ETUDES HYGROPHOTOGRAPHIQUES

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Nous avons décrit dans un autre article intitulé « Une nouvelle technique en physiologie végétale : l'hygrophotographie », le principe et l'application de cette méthode à l'étude de la transpiration végétale et cité les principaux résultats que nous avions pu obtenir déjà dans ce domaine avec elle.

Les méthodes employées jusqu'ici étant peu pratiques et nécessitant la présence d'instruments difficiles à transporter, tels que les balances, ne se prêtaient pas à l'étude de la transpiration dans la nature et en particulier de celle des espèces arborescentes. HUBER en 1927 (1), et tout récemment encore BAILEY, ROTHACHER et CUMMINGS (1952) (2) insistaient avec force sur la grande importance, en écologie, des mesures de la transpiration dans la nature et, afin de permettre cette étude, HUBER (1) avait proposé une méthode de pesée instantanée, dite des « rameaux coupés », qui fut modifiée ensuite par STOCKER (3). Cette méthode, qui permet d'opérer dans la nature, consiste à peser soit les feuilles détachées, soit l'extrémité d'un rameau coupé aussitôt après leur section, puis, après les avoir exposés pendant quelques minutes, convenues d'avance, au lieu du prélèvement, à déterminer leur perte en poids par une nouvelle pesée.

La méthode a été critiquée à plusieurs reprises par les auteurs qui l'ont adoptée, mais les conclusions de leurs critiques n'ont pas été toujours concordantes. Pour SCHRATZ (4), la transpiration diminue rapidement après la section, tandis que pour IVANOFF (5), au contraire, par suite de la rupture de la tension de cohésion, due à la section, à l'intérieur des vaisseaux, la transpiration se trouve augmentée.

C'est aussi l'avis de KAMP (6), de FIRBAS (7) et de GIROUX et SOROCEANU (8). L'augmentation de la transpiration serait sensible surtout de 3 à 5 minutes après la section.

D'autre part, d'après WILLIAMS (9), le choc mécanique amène souvent la fermeture des stomates, ce qui doit entraîner la diminution de la transpiration.

Or, la section étant une opération plus brutale qu'un choc, nous avons voulu étudier par la technique hygrophotographique (10) l'influence de la section sur l'intensité transpiratoire. Ces recherches ayant été commencées en hiver, nous n'avons pu faire nos observations que sur deux espèces d'Haricot, *Ph. vulgaris* et *Ph. multiflorus.*

D'après nos premiers résultats, visibles à l'examen des images hygrophotographiques, l'augmentation de la transpiration, à la suite de la section, semble être générale et bien manifeste.

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DEUXIEME RAPPORT

SOME RECENT ADVANCES IN THE STUDY OF ESSENTIAL MICRONUTRIENTS FOR GREEN PLANTS

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The demonstration of the indispensability of a new micronutrient for plant life is possible only when experimental conditions are so adjusted that the requirement of the element by a given species exceeds the supply. This is generally accomplished by a careful purification of the nutrient medium. It is clear, however, that absolute purification in the sense of removing the last atom of a contaminant initially present is unattainable. Moreover, an essential micronutrient will, by definition, always be contained in the seed or in the mother cell. The reserves of, and the quantitative requirements for, a given micronutrient vary from species to species (1). Advances in the study of micronutrients in plant nutrition may therefore be expected to come from two approaches : 1. Selection of different species having new micronutrient requirements which may still be demonstrated by present techniques and 2. increasing for any given species the refinements of experimental technique to a point where hitherto undetected nutritional requirements come to light.

The present communication reports some results from the application of both of these approaches to the study of the micronutrient requirements of a green alga, *Scenedesmus obliquus*. Green algae (Chlorophyceae) possess certain distinct advantages for micronutrient research. Their fundamental metabolism is similar to that of higher plants and they can be grown much more readily with maximum control of environmental variables. Of particular significance is their lack of specialized cells and organs. Each viable algal daughter cell must therefore contain all of the inorganic constituents essential for metabolism. Rapid reproduction by cell division would, in the absence of an added supply, quickly dilute below a level critical for normal cell function, the concentration of an essential micronutrient initially present in abundance in the mother cell or derived from a residual impurity in a purified nutrient medium.

The extension to algal nutrition of the experimental techniques (2, 3) developed for the study of micronutrient requirements of higher plants yielded evidence for the essentiality of molybdenum, whose requirement by Chlorophyceae, as distinct from blue-green algae and higher plants, was hitherto unknown (4, 5). It was found that in *Chlorella pyrenoidosa* molybdenum deficiency resulted in a marked reduction of chlorophyll content (6). For *Scenedesmus obliquus* molybdenum was established as an essential element according to the criteria of essentiality formulated for higher plants (1) : (a) without the addition of molybdenum to the purified nutrient medium growth as measured by dry weight (Fig. 1), fresh weight (volume of packed cells) and cell count, was reduced to half on the third day and practically ceased on the sixth day ; (b) the deficiency was specific for molybdenum and was corrected by the addition of this element (Fig. 2) but not by that of 22 others (the remaining elements in the *B*7 and *C*13 solutions (8)) ; (c) the participation of molybdenum in *at least* one vital metabolic function of the alga was reflected in the marked reduction in the chlorophyll content of molybdenum-deficient cells (Fig. 3a and 3b).

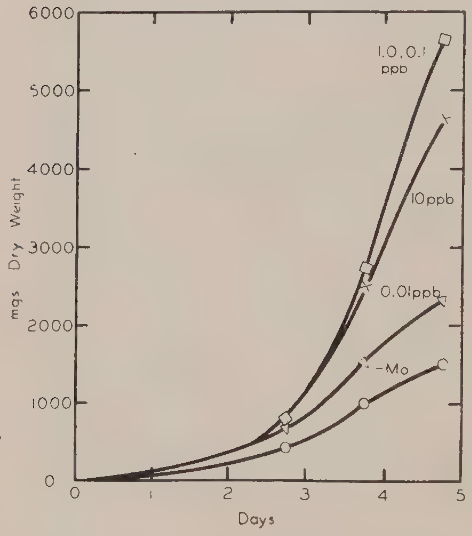

Fig. 1. — Effect of different concentrations of molybdenum on growth of *Scenedesmus obliquus*, expressed as mgs. dry weight per liter of nutrient solution. Lowermost curve : no molybdenum added; upper curves represent effects of increasing concentrations of molybdenum. I ppb = 10^{-6}g. Mo per liter of nutrient solution.

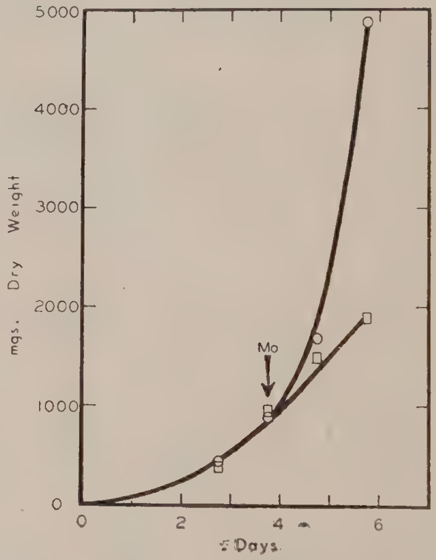

Fig. 2. — Effect of adding molybdenum on the growth of a molybdenum-deficient culture expressed as mgs. dry weight per liter of nutrient solution. At time indicated by arrow 8 micrograms of Mo as (Mo 0_3) per liter of nutrient solution was added to one of two deficient cultures.

The molybdenum requirement of Scenedesmus was found to be low. The addition of 10^{-8} g. Mo per liter of nutrient solution (1 part in 100,000 million) approximately doubled and the addition of 10^{-7} g. Mo per liter quadrupled the yield of algae in six days. No further increase in growth was obtained from higher concentrations of molybdenum (Fig. 1). The unicellular character of growth of the alga has permitted us for the first time, to give a quantitative expression to the molybdenum requirement in terms of atoms per cell (Table 1). This was done by computing the number of atoms of Mo per cell in the culture grown with a restricted molybdenum supply (10^{-8} g. Mo per liter). The number of cells in the minus-molybdenum culture served as a blank, since it represented growth at the expense of the molybdenum in the inoculum and in the residual impurities of the nutrient medium. By subtracting this blank from the number of cells in the culture to which molybdenum was added, a net cell count representing growth as a function of molybdenum concentration was obtained. The computations in Table 1 give maximum values, since it was assumed that all the molybdenum supplied was absorbed. It is seen from Table 1 that during the period of most rapid growth the supply of molybdenum has remained above 3000 atoms per cell. On the sixth day when deficiency was manifest in the Mo-restricted cultures, the supply of molybdenum dropped to about 1300 atoms per cell.

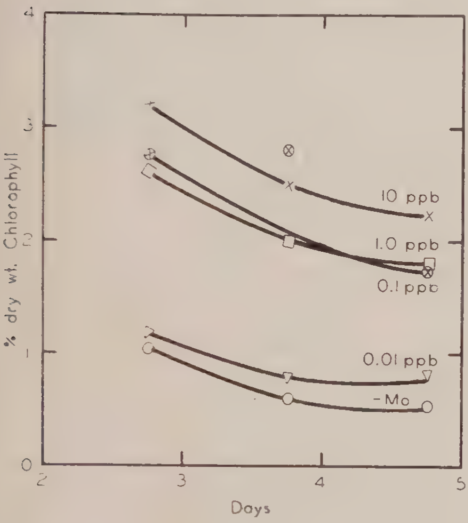

Fig. 3 a. — Effect of molybdenum concentration in the nutrient solution on the chlorophyll content. The concentrations of molybdenum correspond to those given in the legend of fig. 1.

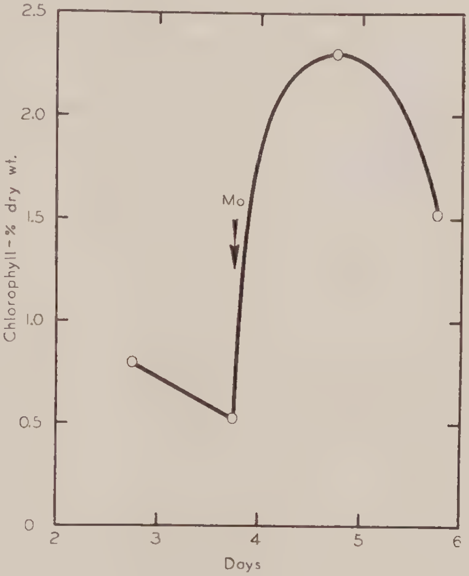

Fig. 3 b. — Effect of adding molybdenum on the chlorophyll content of a molybdenum-deficient culture. Molybdenum was added as described in the legend of fig. 2.

Since computations of this sort may be subject to considerable error, it seemed desirable to check the previous conclusions in another manner :

by computing the number of atoms per cell in a culture grown with an adequate supply of molybdenum (10^{-7} g. per liter). Table 1 shows that during the most vigorous growth period of this culture the supply of molybdenum remained above 3000 atoms per cell. On the fifth day when the density of growth was approaching the terminal level under our light conditions, the supply of molybdenum was still above 2000 atoms per cell. It appears therefore, that to insure vigorous growth of *Scenedesmus obliquus* the supply of molybdenum should be maintained above 3000 atoms per cell.

As shown in Fig. 3a the molybdenum-deficient cells had a markedly low chlorophyll content. The chlorophyll concentration in the cells varied, on a dry weight basis, from approximately 0.5 per cent in the deficient cells to 2-3 per cent in the cells supplied with adequate molybdenum. Intermediate values were obtained with a restricted molybdenum supply. The addition of molybdenum to the deficient cells increased the chlorophyll concentration from 0.5 to over 2 per cent (Fig. 3b) within one day. This profound effect of molybdenum on chlorophyll synthesis, whether direct or indirect, provides further evidence for the importance of this element in the metabolism of the cell. The association of molybdenum with nitrogen metabolism (9) and the nitrate-reductase enzyme (10) have previously come to light.

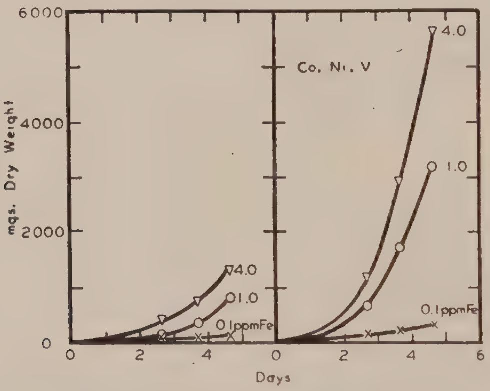

Fig. 4. — Effect of a mixture of cobalt, nickel and vanadium (B3 solution) on growth in a purified medium at three levels of iron : 0.1, 1.0 and 4.0 p.p.m. 1 p.p.m. = 10^{-3} g. per liter). Left. - growth (mgs. dry wt. per liter of nutrient solution) without the addition of the B3 solution. Right - the effect of adding the B3 solution. The B3 solution supplied 10 micrograms per liter of Co, Ni, and V as $Co(NO_3)_2$, $NiSO_4$, and NH_4VO_3, respectively.

In these as in our previous experiments which yielded evidence for the essentiality of molybdenum for higher plants (2), the purification of the nutrient solution was limited to macronutrients and water. Iron, which was being added in micronutrient concentrations, was not purified on the assumption that it was, on a quantitative basis, a negligible source of impurities. With *Scenedesmus* however, a marked increase in growth was observed with increasing iron concentrations in the presence of all the known essential micronutrients for higher plants (Mn, B, Cu, Zn and Mo). It seemed possible that this resulted from some unknown essential micronutrient (11) commonly present as an impurity in iron salts.

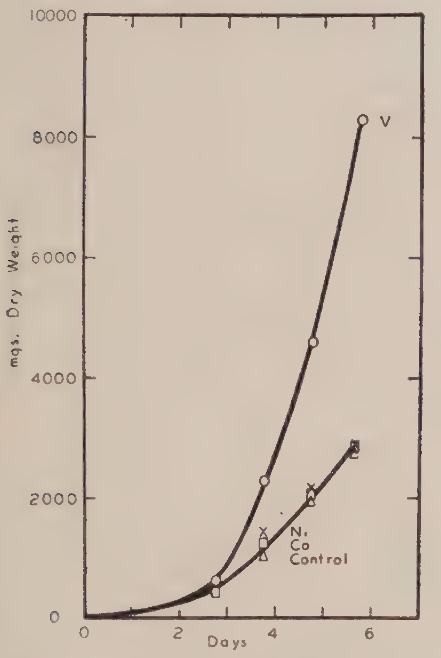

Fig. 5. — Effect of adding singly nickel, cobalt and vanadium (supplied from sources and in amounts given in legend to Fig. 4) on growth (mgs. dry wt. per liter of nutrient solution). 1 p.p.m. Fe was supplied as $FeCl_3$.

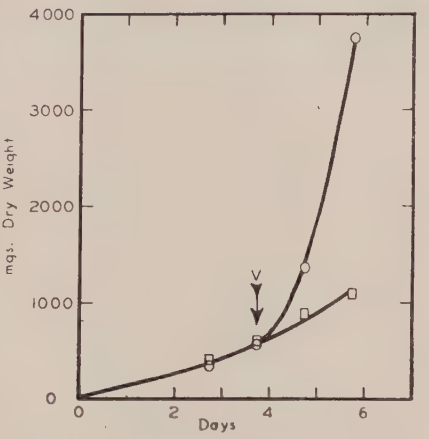

Fig. 6. — Effect of adding vanadium on growth of vanadium-deficient cultures. At the time indicated by arrow 20 micrograms of V (as NH_4VO_3) were added per liter of nutrient solution. Growth is expressed in milligrams dry weight per liter of nutrient solution. 1 p.p.m. Fe was supplied as $FeCl_3$.

This hypothesis was tested by purifying ferric chloride, as a source of iron, by a procedure (12) particularly effective for the removal of cobalt but also likely to remove or reduce other metal impurities such as nickel and vanadium. The algae were grown at three levels of iron supply : 0.1, 1.0 and 4.0 mgs. of iron per liter of nutrient solution. To one series of cultures was added a supplementary solution (*B*3) which provided 10 micrograms per liter each of cobalt, nickel and vanadium. The results are shown in Fig. 4. In this, as in all subsequent experiments, measurements of growth by dry weight (shown here), and by the additional criteria of fresh weight (packed cell volume), cell count and chlorophyll content, gave concordant results.

0.1 mg. iron per liter was clearly insufficient for growth regardless of the addition of the supplementary solution *B*3. However, at 1.0 and 4.0 mg. iron, a marked increase in growth has resulted from the addition of a mixture of cobalt, nickel and vanadium. At iron concentrations adequate for vigorous growth, that is, 1 mg. or more per liter, the growth of algae was distinctly limited by the insufficiency of at least one of the three elements in the *B*3 solutions. Fig. 5 shows that vanadium was the effective element. The addition of cobalt or nickel gave no measurable increase in growth. In another experiment, four parallel cultures were grown in a purified medium without the addition of vanadium. On the fourth day, when a deficiency was apparent, the addition of vanadium to two of the cultures was followed by a sharp increase in growth (Fig. 6).

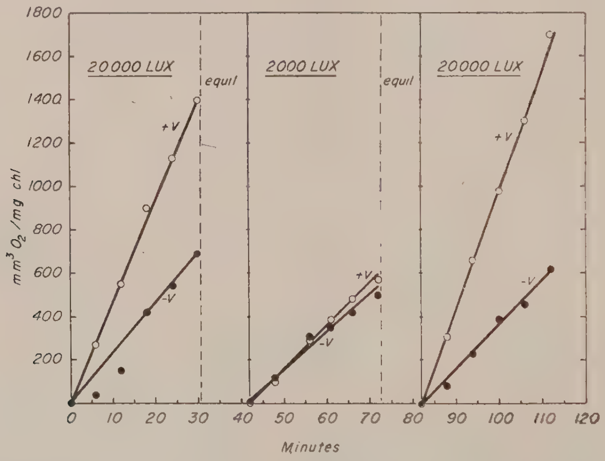

Fig. 7. — Effect of vanadium on photosynthesis at different light intensities. Three-day old cultures grown in either — V or + V nutrient solutions were used. Oxygen evolution was measured continuously with the light intensity changing at the time indicated by the broken lines. A period of equilibration followed each change of light intensity.

Sixteen other elements, titanium, chromium, tungsten, aluminium, arsenic, cadmium, strontium, mercury, lead, lithium, rubidium, bromine, iodine, fluorine, selenium and beryllium (the remaining elements in the *B*7 and *C* 13 solutions (8)), added jointly to the vanadium-deficient cultures, failed to substitute for vanadium.

Although there is no evidence at present for the indispensability of vanadium for other green plants it appears likely that this element will be shown to be generally required by plants. The essentiality of molybdenum was also first established for only one species (2) by criteria similar to those used for vanadium. **Bertrand** (13) has already found that vanadium is widely distributed in plants. Of sixty-two species analyzed by him all contained this element.

The search for a physiological role of vanadium yielded evidence (14) that it is involved in *at least* one physiological process, photosynthesis. This is illustrated in Fig. 7, which is representative of many similar experiments. Photosynthesis of plus and minus vanadium plants was studied at a high (20,000 Lux) and a low light intensity (2000 Lux). The rate of photosynthesis, expressed on a unit *chlorophyll* basis, was twice as high in the plus vanadium cells as in the minus vanadium cells, but only in strong light. In weak light there was no great difference between the two cultures in the rate of photosynthesis expressed on a chlorophyll basis. These results suggest that vanadium deficiency may not primarily interfere with the photochemical events but rather with the dark enzymatic reactions, which limit the overall rate of photosynthesis at high light intensity.

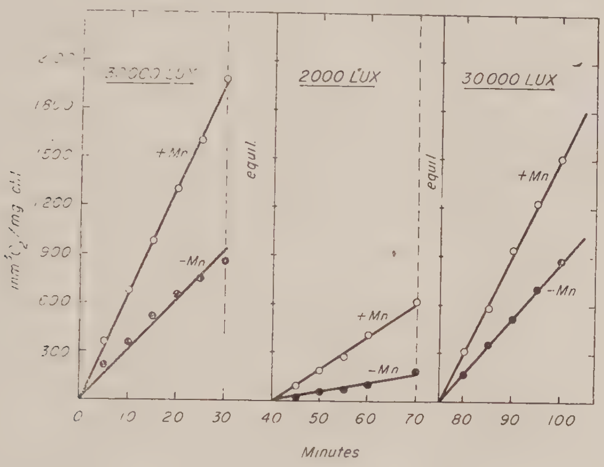

Fig. 8. — Effect of manganese on photosynthesis at different light intensities. Experimental details as in legend of fig. 7.

The effects of vanadium on photosynthesis invite an interesting comparison with those of manganese and molybdenum. Manganese deficiency was found (15) to reduce the rate of photosynthesis in both weak and strong light (Fig. 8), suggesting that manganese may be involved in the photochemical reactions. Manganese deficiency, unlike that of molybdenum, and to a lesser extent vanadium, did not reduce significantly the chlorophyll content of the cells. Total growth as measured by cell count and dry weight was arrested but the chlorophyll content of the manganese-deficient cells was the same as that of the controls.

Molybdenum deficiency, as shown previously (Fig. 3), resulted in a marked reduction in chlorophyll content and hence in the rate of photosynthesis per cell. However, the rate of photosynthesis per unit chlorophyll remained essentially unaltered. A tentative conclusion can be drawn that reactions catalyzed by molybdenum are concerned with the photosynthetic process only indirectly, through their effect on chlorophyll synthesis.

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CALCIUM TRANSLOCATION AND ASCORBIC ACID STATUS IN PEAS

by Herbert JONAS

Peas (*Pisum sativum*, var. Alaska Bush) were grown during the summer in the greenhouse with aerated Hoagland's solution (18.71 m. e. of salt liter, pH 5.5). Ca was withdrawn from all plants at full flowering 34 days after germination and substituted by NaNO$_3$. This was replaced 12 days later by 28.5 m. e. of CaCl$_2$ with 3.30 μc of Ca^{45}. All plants were harvested 4-8 days later when roots and stems of the -Ca plants had striking deficiency symptons. Quantitative determinations showed 10x more Ca* in the lower and middle stems and branches than in their leaves and much more Ca* in the carpels than in their seeds. The roots were high in Ca*. The average rate Ca* accumulation in each part of all plants was inversely proportional to their total Ca content. In particular, starvation loss of total Ca in the middle stem sections caused a higher rate of Ca replacement. The carpels acquired much Ca, probably at the expense of nearby stems and leaves. The roots of +Ca plants contained less Ca than any other organ and less than the controls. Continued starvation apparently depleted the middle and top sections of the plants of Ca, while added Ca seemed to even out the Ca concentrations of all plant sections. — The tissue sections were analyzed for reduced ascorbic acid (A.A.) prior to the Ca determinations. The tissue sections of plants grown under continuous Ca starvation had A.A. contents in proportion to those of Ca. The lower and upper stems and leaves had peak Ca and A.A. contents while the middle sections and the roots were low in both. About 80 m. e. of Ca corresponded to 1 m. e. of A.A. The fruit Ca was inversely proportional to A.A., probably due to deficient fruit initiation. The vegetative tissue sections of plants furnished with Ca* labelled CaCl$_2$ also indicated a trend of proportionality between total Ca and A.A. Between 80 to 110 m. e. of Ca corresponded to 1 m. e. of A.A. But the Ca : A.A. relation in the individual plant levels was irregular. Generally the lower and top leaves were high in A.A. while Ca was high in or near these regions. Definite minima of total Ca and A.A. occurred in the roots and fruits, especially in the seeds. The addition of Ca* seemed to be the cause of these sectional irregularities. An inverse relationship between Ca* and A.A. was superimposed on the total Ca : A.A. proportionality. Wherever Ca* was low due to a high residual stable Ca content the concentration of A.A. rose (lower leaves, top tissues). Again, high Ca* concentrations corresponded to reduced A.A. formation. About 4×10^{-4} to 2×10^{-3} m. e. of Ca*/g of fresh weight/10 plants produced a maximum of 3 to 6×10^{-2} m. e. of A.A. Ca* concentrations below or above this threshold were less inducive to A.A. production. This maximum corresponded to a Ca* : total Ca ratio of 10^{-4} to 10^{-2}. (This abstract is based on *Nucl. Sc. Abstr.*, **6**, 1980, 1952, and *First Quarterly Report*, Biol. Div., O.R.N.L., 1952.)

MINERAL ABSORPTION THROUGH VARIOUS REGIONS OF ROOTS

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Chemical analyses of barley roots indicated that the highest concentrations of phosphorus and other mineral elements occur in the meristematic region or at least very near the root tip. When the roots were grown for a week in dilute nutrient solution containing P^{32}, the distribution of P^{32} closely paralleled the distribution of total P as determined by chemical analysis. The concentration was highest in the apical 2 mm., lowest in the region 2 to 10 mm. behind the apex, and intermediate in the root hair zone 20 to 80 mm. behind the tip. After only 2 hrs. in a solution containing P^{32}, however, more P^{32} had been absorbed per mm. in the root hair zone, several cm. behind the tip, than in the meristematic region. Furthermore, considerable P^{32} was translocated away from the root hair zone to the shoot, but very little was translocated out of the root tip.

These results suggested that the region of greatest eventual accumulation in the root is not the region of most rapid absorption and translocation to the shoot. It seemed probable that accumulation in the cells of the meristematic region is entirely distinct from absorption by the plant as a whole, which might occur chiefly in more mature regions of the roots.

To measure absorption in various regions of roots, 5-day old barley seedlings were immersed in full strength nutrient solutions containing radioactive isotopes of P, I, Rb, Ca, or S for 2.5 hours. The seedlings were then washed, dried, and autoradiograms were made. The roots were cut into segments and the radioactivity of each segment was measured. Absorption of all isotopes occurred along the entire length of the root, but there were large variations in the amounts absorbed in various regions. Absorption of all isotopes was high in the first 2 mm. behind the tip which included dividing and enlarging cells. It was generally lower 2 to 6 mm. behind the tip where cell elongation occurs most rapidly, except that absorption of S^{35} was high in this region. Absorption of P^{32} and I^{131} per mm. of length was higher in the younger part of the root hair zone than in the root tip, but absorption of Rb^{86}, Ca^{45}, and S^{35} was slightly lower per mm. in the root hair zone than in the root tip, although the area over which absorption occurred was of course much greater in the root hair zone. In slowly growing roots the absorbing zone occurred nearer the root tip, probably because differentiation of xylem occurs nearer the tip.

Translocation from various regions of individual attached roots was also studied. Three mm. segments of roots were inclosed in absorption cells in such a manner that an isotope could be supplied to the inclosed portion of the root while the remainder of that root and the other roots of the seedling were immersed in nonradioactive nutrient solution. The absorption cells were attached at the tips and about 10, 30, or 60 mm. behind the tips. The isotopes listed previously were supplied to the roots for 6 hrs. at 25°C. When supplied to the root tips heavy absorption of all isotopes occurred, but less than 5 per cent of absorbed isotope was translocated to other parts of the seedling. At least 3 times as much of the absorbed isotope was translocated away from the zone of cell enlargement and differentiation 10 mm. behind the tip as from the tip. Upward translocation was greatest from the 30 mm. region, ranging from 11 to 34 per cent of the total isotope absorbed, and translocation from the 60 mm. zone was almost as great. Downward translocation of all isotopes occurred when supplied to regions above the root tips. Considerable P^{32} and S^{35} moved down and accumulated in the tips, but the other isotopes stopped short of the tips.

The results of these experiments indicate that although the meristematic region accumulates large amounts of minerals very little is translocated to other parts of the plant from it, probably because no path for translocation exists until the xylem becomes differentiated. Apparently most of the minerals which are translocated out of the roots are absorbed in the root hair zone. The exact location of the zone of most rapid absorption from the plant as a whole probably depends on the rate of differentiation and maturation of the roots, but our data indicate that it is in the region of differentiation rather than in the meristematic region.

INFLUENCE OF MAGNESIUM ON THE UPTAKE OF MANGANESE

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Excess of manganese as a source of injury for a number of crop plants has been recognized as a possible component of the soil acidity complex ; these investigations have been reported in former papers (1, 2, 3). *Phaseolus vulgaris* (varieties brown beans and French beans) appeared among the most sensitive crops when tested on experimental plots, the unlimed of which were highly acid. During the experiments carried on in successive years beans and potatoes have always been grown along with the other crops under investigation. In 1949 in the most acid plots magnesium deficiency was very conspicuous in young potato plants and a top dressing with $MgSO_4$ was applied to halves of those potato crops and to bean crops in the same plots. At that moment the disorder caused by Mn excess was very conspicuous in the beans. Some weeks later the beans on the Mg halves had developed much better than those without Mg, although in the older leaves of the former symptoms of Mn excess still occurred. When the Mn content in corresponding bean lots was estimated, it appeared to be much lower in the better developed Mg plants. In all following years half of each plot in the experimental series received a top dressing with $MgSO_4$ and a same reducing effect of Mg on the uptake of Mn could be ascertained. The largest differences noted calculated as ppm Mn on the dry substance were : Brown beans —Mg 2664, +Mg 1164 ; French beans —Mg 2278, +Mg 1392. By means of growing beans in von der Crone solution varying in content of $MgSO_4$ the antagonistic effect of Mg on the uptake of Mn could be corroborated.

Potatoes which up to 1950 never had shown any visible sign of Mn injury showed in that year on the halves of the acid plots which had not been dressed with $MgSO_4$ a very characteristic disorder. Elongated, sunken, dark streaks appeared at the basis of the stems and later also higher up the stems. In the leaves minute dark spots appeared leading to collapse of the leaf tissue. Plants remained stunted. The Mn content more especially of the stems of such plants was much higher than in the corresponding $MgSO_4$ treated plants (e. g. —Mg 2849, +Mg 1424). By means of solution cultures supplied with 50 or 100 mg Mn/L in which a similar disorder could be induced in potatoes, Mn excess could be recognized as the cause. By varying the level of Mg in these cultures the Mn uptake, however, could not be influenced, the development of the stunted plants in the nutrient solutions varying greatly.

The occurrence of Mg — Mn antagonism had been studied in other economic plants grown on the experimental plots. 1951 and 1952 have been favorable for these investigations.

In *soy beans* this antagonism could be ascertained by visible symptoms and by difference in the Mn content of + and —Mg plants. A lowering of the Mg level in the nutrient solution did not result, however, in an increasing uptake of Mn.

Vicia sativa, although very sensitive for Mn injury did not show any favorable action of Mg either on injury in the field or on the Mn content of the plants.

Flax, although very sensitive for soil acidity, is a crop for which no injurious action of Mn could be ascertained. A dressing with $MgSO_4$ did not result in any reduction of the uptake of Mn.

In *serradella* and *lupine*, thriving well even in the most acid plots, where the Mn content of the plants was very high (serradella max. 2030, lupine 2780), the $MgSO_4$ supplied did not influence the uptake of Mn.

The cereals tested were all very insensitive for Mn in the field, owing to their much lower uptake of Mn. The highest level was in *barley* in an unlimed plot without $MgSO_4$: here the young plants bore minute dark spots due to excess of Mn, but recovered at early date. In the acid plots, however, the uptake of Mn was lower in the Mg treated crops. In nutrient solutions with a high content of Mn (50 mg Mn/L) a very low Mg level (0 or 50 mg $MgSO_4$/L) gave rise to plants with a much higher content of Mn than in solutions containing 200 mg $MgSO_4$/L. Max. differences : *barley* (0) 1369-(200) 776 ; *wheat* (0) 743-(200) 461 ; *oats* (0) 1176-(200) 774 ; *rye* (50) 1121-(200) 721. Although any trouble in the field by Mn excess is hardly to be expected in cereals, the Mg — Mn antagonism exists here as well.

It might be conceived that Mg acted on the availibility of Mn in the soil, the plant playing only a passive part. Soil percolates from corresponding + and — Mg plot halves have been compared as to their content of Mn. No influence on the availiblity of Mn could be noted.

Apparently for various crop plants an antagonism between Mn and Mg has to be reckoned with in field practice, although for other crop plants no such antagonism could be ascertained.

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MOLYBDENUM AND NITRATE REDUCTASE IN *NEUROSPORA CRASSA*

by D. J. D. Nicholas and Alvin Nason

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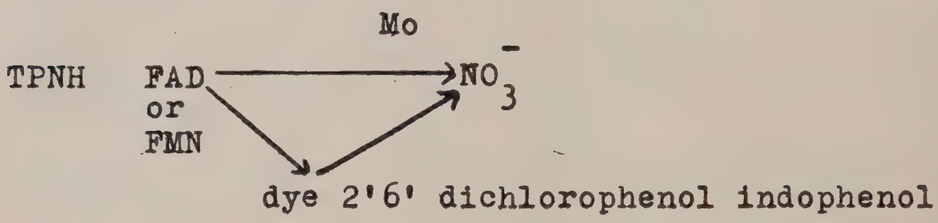
It is well known that a molybdenum deficiency in higher plants and fungi may result in an accumulation of nitrate in the tissues. Molybdenum has now been identified as the metal constituent of nitrate reductase in *Neurospora crassa* thus demonstrating a rôle for the micronutrient in nitrogen metabolism. The enzyme is a flavoprotein containing flavin adenine dinucleotide (FAD) and requiring reduced triphosphopyridine nucleotide (TPNH) as the specific electron donor. Flavin mononucleotide (FMN) can substitute for FAD in the above reaction. The mechanism of electron transport has been determined to be :



Nitrate reductase purified approximately 100-fold was used in the following experiments. TPNH reduces FMN enzymatically and the electron can be further transferred to a dye by nitrate reductase. This part of the system is not cyanide sensitive and can be mediated successfully by a molybdenum free enzyme. The addition of FMN or FAD increases the rate of flavin or dye reduction. Reduced FMN or leucodye does not reduce nitrate enzymatically in the presence of 10^{-3}M cyanide ; neither will a molybdenum free enzyme mediate this electron transfer. The addition of molybdenum to the Mo free enzyme restores its capacity to transfer electrons from the reduced flavin or leucodye to nitrate ; whereas the addition of other micronutrients is without effect. The addition of flavin does not appear to stimulate this part of the system so that the metal occurs after the flavin in the over-all enzymatic reaction. Further details of the reduction mechanism of nitrate reductase will be discussed.

TROISIEME RAPPORT

THE EFFECTS OF MINERAL DEFICIENCIES ON CROP PLANTS

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Interest in the pathological effects of mineral deficiencies in crop plants has been stimulated during the past three decades by the growing recognition of the importance of the trace elements in crop production. Prior to that time manurial practices, so far as mineral elements were concerned, had been based almost wholly on the established effects of nitrogen, phosphorus and potassium and of lime in increasing yields, which inevitably focussed attention on the importance of these three elements and of calcium in plant nutrition. This outlook, however, was altered by the recognition of the special needs of certain crops for other elements, such as tobacco for magnesium, sugar beet and swedes for boron, oats and peas for manganese and fruit crops for iron. Following these discoveries the importance of other elements in practical crop production has been demonstrated, e. g. sulphur for tea, cotton and lucerne, copper for cereals, lettuce and tree fruits, zinc for citrus and other tree fruits and for cereals and maize, and most recently molybdenum for legumes, oat and *Brassica* crops.

At the present time, twelve mineral elements have been proved to be essential for the growth of higher plants, taking the criteria suggested by Arnon (1) as defining an essential element : viz. nitrogen, phosphorus, calcium, magnesium, potassium, sulphur, iron, manganese, boron, copper, zinc and molybdenum. If the recent findings relating to cobalt and vanadium (2, 3) are verified, it will be necessary to add these elements to the list.

In addition it is necessary to consider a number of so-called beneficial elements to those above, particularly sodium and chlorine, as affecting yields and the health of crop plants.

It should be noted that all the twelve essential elements are of practical importance in crop production, and deficiencies of each have been identified as causing pathological conditions — sometimes called mineral disorders — of field crops.

Sodium has also been shown to be of great benefit to crops such as sugar beet, various Brassicae and cotton, and pathological effects resulting from the deficiency have been described for some of these crops (4, 5, 6).

In opening this discussion it is my intention to bring to your notice the main effects produced in crop plants of deficiencies of the above elements, and I propose to do this chiefly by means of illustrations of specimens grown either in sand culture or in the field.

For more detailed descriptions I would refer you to my book, *The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms* (6).

Effects of Mineral Deficiencies.

Nitrogen : Growth of shoots and roots is severely restricted ; shoots are upright, thin and stiff ; leaves are small, pale green and tend to develop high tints of yellow, orange, red and purple ; defoliation is early and begins at the older leaves ; lateral shoots and tillers are few and lateral buds tend to be suppressed ; blossom buds are reduced in number, and yields of grain and fruits are small. Growth processes in the spring, such as opening of buds, leaves and flowers, are retarded.

Phosphorus : The general effects on shoot growth, leaf size, defoliation and on the development of lateral buds, shoots, tillers and blossom buds are similar to those described for nitrogen deficiency. Leaf tints may also be similar to those for nitrogen deficiency, but incline more to purple and bronze shades, and for some plants tinting is absent and instead marginal leaf scorch or spotting effects are developed. The colour of phosphorus deficient leaves is usually a dull bluish green, which contrasts with the pale green of nitrogen deficient plants. Fruits tend to be of high acidity.

Calcium : The outstanding symptoms of calcium deficiency are shown at the growing points of shoots, in young leaves and in the roots.

Death of growing points is common, followed by dying-back of shoots ; young leaves become distorted and develop scorched areas near the tips and margins ; leaf mesophyll tissues, stems, petioles and pedicels often collapse, resulting in pronounced wilting effects ; roots are poorly developed, lack fibre, and are often short and blunt, and of gelatinous appearance ; flowers and fruits may be malformed or fail to develop, and tuber formation is severely restricted.

In the field it may be necessary to distinguish between the effects of calcium deficiency and the gross effects of soil acidity, in which several factors are concerned.

Magnesium : The effects of magnesium deficiency are most evident in the leaves of plants, where they may be most spectacular. The leaf symptoms vary greatly on different plants and include chlorosis, high tinting and interveinal and marginal necrosis. These effects generally develop in well defined and characteristic patterns, the central interveinal pattern being very common over a wide range of plants. Severe and premature defoliation, following rapidly on the development of leaf symptoms, is an outstanding characteristic of magnesium deficiency, and abscission often occurs without the usual signs of progressive leaf senescence. The leaf symptoms, like those of nitrogen and phosphorus deficiencies, develop first on the older parts of the plants and spread progressively to the younger growths. Dwarfing and other effects on growth follow from the premature defoliation.

Potassium : The growth characters of potassium deficient plants vary somewhat according to the severity of the deficiency.

In instances of mild deficiency shoots may be rather thin, in relation to length, whereas when the deficiency is acute growths tend to be short, with short internodes, and die-backs may develop ; in cereals, there may be a tendency to tiller excessively with the formation of relatively few flowering stems. Leaves of potassium deficient plants are generally bluish green in colour, but may exhibit some interveinal chlorosis, and they may show rolling and distortion on broadleaved plants ; tip and marginal leaf scorch effects are common and some plants develop characteristic spotting patterns. Flowering is not severely affected in the initial stages of the deficiency, but later both flowering and fruiting may be drastically reduced. Fruits are often markedly sub-acid.

In some sand culture and field experiments the opening of buds and blossoms in spring has tended to be advanced.

Sulphur : The general effects of sulphur deficiency are similar to those of nitrogen deficiency. Shoot growth is restricted, growth erect and shoots are stiff and thin. Leaves are pale and may show marked yellowing and chlorosis, as in tea, or high orange or red tints.

Iron : The characteristic effect of this deficiency is a chlorotic condition of the terminal leaves of shoots, this condition decreasing progressively from young to older leaves. The chlorotic patterns on the leaves are often characteristic, especially in the early stages, a common feature being that the complete network of veins remains green and clearly outlined in the chlorotic tissue.

In some plants, e. g. Brassicae, the pattern may take the form of mottling, and in cereals comprises an interveinal striping. Chlorotic leaves, in the later stages, usually develop brown necrotic patches, particularly near the margins, as in fruit trees, and death of growing points and shoot die-back is common. Fruits may be brightly coloured and be deficient in chlorophyll in green surfaces, e. g. in the apple.

Manganese : The visual effects of this deficiency comprise several distinct forms of symptoms, including chlorosis and necrotic spotting and blotching of leaves, and necrosis of the cotyledons of the seeds of legumes. The chlorotic patterns vary for different types of plants : a common form on many fruit plants and shrubs is a V-shaped interveinal pattern, which begins near the leaf margin and spreads towards the midrib, often obliterating the fine network of veins ; in Brassicae a mottled pattern is developed and leaves may become almost wholly chlorotic ; in sugar beet and other related plants a characteristic mottled pattern is developed, later followed by necrosis of the mottled areas, known as « speckled yellows ». In tomatoes the chlorosis comprises a very fine speckle, the specks later becoming necrotic.

Spotting patterns are often well developed on potatoes, the spots usually following the lines of the veins. The blotch form of necrosis is typified in the so-called « grey speck » of oats and in other cereals.

Necrosis of the cotyledons of the seeds is most clearly shown in peas, but may be produced in many species of bean in sand culture.

Boron : Boron deficiency effects are prominent in meristematic tissues in the form of die-backs of growing points and distortion of organs, which may be accompanied by chlorosis and pigmentation of leaves. Stems may be stiff and brittle.

The various effects that may occur are typified by the descriptive names given to the pathological conditions that result in various crop plants from the deficiency, e. g. « Heart Rot » of sugar beet and mangold, « Canker » of garden beet, « Brown Heart » of swede and turnip, « Hollow Stem » of cauliflower, « Cracked Stem » of celery, Alfalfa « Yellows », « Drought Spot », « Cork » and « Corky Core » of apple, « Hard Fruit » of Citrus and « Tip Sickness » of tobacco.

The various distortions as shown for beet and cabbage appear to result from an increased activity of cell formation, accompanied by a lack of tissue differentiation (6).

Copper : Deficiency of copper, like calcium, iron and boron, is most evident in young growths. The most common effects are death of growing points of shoots, followed by progressive die-back to the older tissues, chlorosis and wilting of foliage, distortion of shoots and gumming, excess tillering following die-back in cereals, with failure of the plants to form grain.

These various effects are typified in Exanthema and Summer Die-back of tree fruits, gumming of *Prunus* sp., and « Yellow Tip » or « Withertip » or « Reclamation Disease » of cereals.

Zinc : The main effects of zinc deficiency are shown as chlorosis and reduced size of leaves, together with the development of purple tinting in some plants, and a marked shortening of internode lengths in stems and shoots. The chlorotic pattern may be very distinctive, as in « Mottle Leaf » of Citrus, and the reduction in leaf size as in the « Little Leaf » condition of apple, « Sickle Leaf » of Cacao and the small-leaved plants that result in flax and clovers. Tinting is typified in « Bronzing » of tung and is shown also in cereals.

Molybdenum : Molybdenum has now been shown in sand culture to be essential for a large number of crop plants. The main effects in Brassicae are reduction in the leaf size of younger leaves, failure of the leaf laminae to develop (e. g. « Whiptail » in cauliflower), distortion of young growths and ultimate death of the growing points; areas of dead tissue may also develop on leaves, beginning near the margins, and there may be some chlorotic mottling. Tomatoes and lettuce leaves develop chlorotic mottling, and those of tomatoes show pronounced inward rolling of the marginal areas.

In legumes, the deficiency in the field may be evidenced as a deficiency of nitrogen, due to the failure of the nodule organisms to « fix » nitrogen. If a suitable nitrogen supply is ensured to legumes in sand culture, the deficiency shows mainly in the death of leaf tissue.

Sodium : In plants requiring sodium for maximum growth, deficiency of the element is shown in the leaves, which tend to be thin, dark green in colour and lacking in lustre, with a tendency to grow out horizontally from the crown of the plant and to wilt readily in drought. Marginal leaf scorch, similar to that resulting from potassium deficiency in these plants, may develop.

Conclusion

In concluding this paper I should like to draw attention to what I consider to be two urgent needs in mineral deficiency investigations at the present time. These are for the detailed histological examination of the organs of plants growing under deficiency conditions, such as have been carried out by Walker *et al.* (7) on boron deficient garden beet and cabbage, and for biochemical studies to determine the roles of the various elements in vital metabolic processes.

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COPPER AND ZINC DEFICIENCIES IN SOILS AND FRUIT TREES

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Floyd was the first to show that a die-back or « exanthema » condition in *citrus* was due to copper deficiency. Since then copper deficiency has been observed in apple, pear, plum, prune, olive, avocado and tung.

During the 1948 season « summer die-back » symptoms were observed in shoots of apple and pear in the National Fruit Trials at Wisley, England. These symptoms were similar to those of copper deficiency as described by **Anderssen**, **Oserkowsky** and **Dunne** and it was shown that the disease was caused by an acute deficiency of copper in the soil and trees. This was the first recorded instance of copper deficiency in fruit trees in England although cereal crops in East Anglia were known to suffer from copper deficiency. Since 1948 **Jones** has shown that a die-back condition of pears can be corrected by foliage sprays of copper sulphate. Copper deficiency has also been diagnosed at a further eight centres in the S.W. of England occurring in apple, pear, almond and various ornamentals.

Chandler *et al* were the first to recognise and correct zinc deficiency in fruit trees. Since then it has been diagnosed in other fruit growing areas in America, Australasia, Africa and Europe.

In July 1948, investigations into a serious failure of apple trees at Wisley showed that the main cause of the failure was due to zinc deficiency. Further instances of zinc deficiency have been diagnosed in the S.W. of England occurring in apple, pear, almond, cherry and ornamental crab. Confirmation of these deficiencies has been made by means of chemical and *Aspergillus niger* soil tests, by leaf analysis, and correction by foliage sprays.

Copper and Zinc Status of Soils

The soil at Wisley is a coarse sand and gravel overlying deep gravel. It was originally acid but the pH was raised to 7 by applications of limestone. Other centres investigated are mainly on soils of the Lower Greensand. Chemical and biological tests were carried out on these soils and the results are given in Tables I, II and III.

Table I gives data for the Wisley soil. The results show a close agreement between exchangeable zinc, as determined by the neutral normal ammonium acetate method, and available zinc as determined by the *Aspergillus niger* test. The copper soluble in cold N/10 HCl and the biological

TABLE I

Chemical data for soil from an area at Wisley where fruit trees showed severe zinc and copper deficiencies.

Depth in.	pH	% Organic matter	% P_2O_5 soluble in 1% citric acid	µg per g. of soil (p.p.m.)							
				Exchangeable by N neutral ammonium acetate method. (10 g. soil + 200 ml solvent)		Soluble in N ammonium acetate adjusted to pH 4 with acetic acid. (10 g. soil + 200 ml solvent)		Soluble in cold N/10 hydrochloric acid. (10 g. soil + 100 ml solvent)		*Aspergillus niger* method	
				Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
0 - 6"	7.2	1.29	0.06	Trace	1.31	0.10	8.9	0.56	10.2	0.8	1.5
6 - 12"	7.0	0.83	0.03	"	0.74	0.10	2.9	0.34	4.0	0.4	0.5
12" +	6.8	0.45	0.02	"	0.90	0.07	2.7	0.26	3.6	N.D.	N.D.

TABLE II

Effect of various solvents on the extraction of copper and zinc from a threshold soil. 10 g. soil leached with successive 200 ml volumes of solvent.

Solvent	µg per g. soil (p.p.m.)							
	Copper				Zinc			
	1st extract	2nd extract	3rd extract	Total	1st extract	2nd extract	3rd extract	Total
N. ammonium acetate pH 7	0.44	0.07	0.11	0.62	0.36	nil	nil	0.36
" " " + acetic acid pH 6	-	-	-	-	1.52	0.78	0.52	2.82
" " " " " pH 5	0.43	0.20	0.11	0.74	6.15	0.95	2.45	9.55
" " " " " pH 4	0.92	0.48	0.48	1.88	9.85	1.70	1.35	12.90
N/2 acetic acid	0.36	0.21	0.14	0.71	7.70	2.90	1.40	12.00
N/2 KCl + acetic acid pH 3	0.15	nil	nil	0.15	2.85	3.35	0.90	7.10
*N/10 cold HCl	2.10	-	-	2.10	13.0	-	-	13.00

* 10 g. soil shaken gently with 100 ml N/10 HCl for 1 hr.

TABLE III

The available copper and zinc of soils as determined by the *Aspergillus niger* method (II)

Location	Depth of sample in.	as µg per g. air-dry soil (p.p.m.)		Deficiency symptoms in fruit trees	
		Cu	Zn	Cu	Zn
Wisley (Surrey)	0 - 6"	0.8	1.5	Severe die-back	Severe rosetting
" "	6 - 12"	0.4	0.5		
Wisley "	0 - 6"	1.2	4.0	Slight die-back	Slight rosetting
" "	6 - 12"	0.8	2.0		
Wisley "	0 - 6"	3.5	9.0	Absent	Absent
" "	6 - 12"	2.0	4.0		
Liss (Hants)	0 - 6"	0.8	-	Severe die-back	Absent
	6 - 12"	0.6	-		
Long Ashton (Somerset)	0 - 6"	25.0	30.0	Absent	Absent
" " "	6 - 12"	15.0	20.0		

TABLE IV

The copper and zinc content of healthy and deficient leaves of fruit trees.

Fruit and location	Plant part analysed	Cu as µg per g. dry matter	Symptoms	Zn as µg per g. dry matter	Symptoms
Apple					
Laxton's Pearmain (Wisley)	Leaf	1.4 - 2.3	Shoot die-back	9.6	Normal from deficient tree
Laxton's Pearmain "	"	-	-	1.2	Little leaf and rosetting
Hereford Cross "	"	1.3 - 3.0	Shoot die-back	3.0	" " " "
Worcester Pearmain (Somerset)	"	2.3	" " "	-	-
Worcester Pearmain "	"	5.0	Normal	20-55	Normal
Lane's Prince Albert (Long Ashton)	"	5.1 - 7.9	"	18-30	"
Pear					
Conference (Gloucester)	Leaf	8.0	Normal	22	Normal
Conference (Exeter)	"	1.1	Shoot die-back	-	-
Cherry					
Sweet Cherry (Holland)	Leaf	14.2	No die-back	13.0	Rosetting
Morello Cherry (Exeter)	"	3.1	Shoot die-back	17.6	Slight rosetting
Almond					
Variety unknown (Exeter)	Leaf	3.1	Die-back	41.5	Normal
Variety unknown "	"	-	-	21.6	Little leaf

test values are also in close agreement. It will be noticed that very little copper is extracted by ammonium acetate even when the pH is lowered to 4 by acetic acid. The effect of different solvents on the extraction of copper and zinc is further illustrated in Table II. This soil induced only slight copper and zinc deficiency symptoms in nursery fruit trees.

Other data, not included in this paper, show that the copper extracted by cold N/10 HCl, according to the procedure recommended by WEAR and SOMMER for zinc, agrees closely with the *Aspergillus niger* test values. The threshold level for copper in soil by the N/10 HCl extraction appears to be in the region of 1-2 p.p.m. Cu. The levels for zinc obtained by this method are much higher than those reported by WEAR and SOMMER. Table III shows the relationship between available copper and zinc in soils, and the occurrence of symptoms in fruit trees. These tests indicate that soils containing more than 2 μg (p.p.m.) Cu and 10 μg Zn per g. air-dry soil provide adequate amounts of these two elements for the normal growth of fruit trees. Soils containing less than 2 μg of Zn per g. of soil will induce zinc deficiency symptoms in fruit trees.

Copper and Zinc Status of Healthy and Deficient Leaves of Fruit Trees

From Table IV it will be seen that the threshold level for copper, coinciding with the onset of deficiency symptoms is 5 μg Cu per g. dry matter. Severely affected trees contain only 1 to 2 μg Cu per g. leaf dry matter.

The threshold level for zinc in apple leaves is around 10 μg Zn per g. dry matter. Severely rosetted leaves contain < 5 μg Zn per g. dry matter.

BOLLARD found the zinc content of healthy nectarine and peach leaves to be 10 and 15 p.p.m. in dry matter and deficient leaves to be 7.5 and 5.6 p.p.m. in dry matter respectively.

Corrective Treatments

Dilute cooper sulphate (0.05 % concentration) or any of the proprietary copper fungicides, when applied as a foliage spray before the death of the terminal growing point of the young shoots (July), will prevent summer die-back caused by copper deficiency.

Dormant sprays of 4-5 % copper sulphate are also effective but more expensive. Applications of copper sulphate to the soil at the rate of 25 to 50 lb. per acre have been effective under acid soil conditions, in the second year after application. Soil dressings of copper salts are less effective under alkaline conditions.

Zinc deficiency is readily corrected by the application of a zinc sulphate foliage spray (0.1 % concentration) at petal-fall. Late applications of zinc foliage sprays (July onwards) are not effective until the following season. Dormant sprays of zinc sulphate at 4-5 % concentration will correct the leaf symptoms in the following season and may be used on fruit varieties which are susceptible to foliage sprays. The residual effect of dormant sprays is not so great as from foliage sprays.

MANGANESE DEFICIENCIES IN PLANTS THEIR OCCURRENCE, RECOGNITION, AND CONTROL

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During the past twenty or thirty years, soils unable, for some reason or other, to furnish a sufficiency of manganese to growing crops have been found all over the world. The literature contains many records of manganese deficiency affecting, with greater or less severity, a large variety of crops.

Circumstances of Deficiency. Most of the recorded instances of manganese deficiency have occurred on soils with comparatively high pH (generally 6.5 and over) and rich in organic matter. Under these conditions, soil manganese is rendered unavailable to the plant. A similar effect is sometimes produced by a high water-table. On the other hand, actual waterlogging may have the opposite effect through conversion of oxidised manganese to an available form.

Manganese deficiency has been reported on mineral soils in many vegetable-growing areas, often as a result of excessive application of lime on naturally acid soils. Instances have occurred, too, on hungry, leached, or well-drained sandy soils, sometimes with distinctly acid reaction.

Detection of Manganese Deficiency. Enormous progress has been made in the study of visual symptoms of mineral deficiencies in plants. Largely by using solutions of pure nutrients in sand or water culture, specific symptoms of deficiencies of mineral elements have been induced in many crop plants. These have been photographed and reproduced in black and white and in colours.

Confirmatory or complementary methods of diagnosis include soil analysis, analysis of plant tissue, field and pot culture trials, and the application of nutrients by means of foliage sprays or tissue injections. Rapid chemical tests for both soils and plant tissues have been developed.

Symptoms of Manganese Deficiency. Perhaps the commonest visual symptom of manganese deficiency is chlorosis of the foliage ; symptoms vary in detail. Usually, small chlorotic patches develop between the veins ; their pattern is largely dependent on the shape of the leaf. In gramineae, they are often elongated, producing ' stripes ' or ' streaks '. In broadleaved plants, ' spots ' or ' speckles ', giving a mottling effect, are more common. Necrotic areas may develop. In beans and peas, small brown necrotic spots or cavities appear on the internal surfaces of the seed cotyledons when separated. In peas the foliage itself may be little affected except with a severe deficiency.

Controlling Manganese Deficiency. Several methods are available for increasing the uptake of manganese by growing crops. Choice of method may depend on prevailing soil conditions and on the nature of the crop.

Lowering Soil pH. Where the deficiency is associated with high pH, the general policy is to avoid the application of lime, basic slag, and other alkaline soil amendments, and to employ acid-forming fertilisers such as sulphate of ammonia. Sulphur has been used successfully under some circumstances.

Application of Manganese Compounds to the Soil. Various compounds of manganese, but generally the sulphate, have been applied, usually along with ordinary fertilisers, in the control of manganese deficiency. Amounts of manganese sulphate recommended have ranged from less than 50 lb. per acre (56 kg/ha) for mineral soils up to 400 lb. per acre (448 kg/ha) on strongly alkaline peats. Speedy recovery has in some cases been effected by mixing the manganese sulphate with two or three times its own weight of sulphate of ammonia or with an equal weight of sulphur. Claims have been made that the manganese naturally present in appreciable amount in certain fertilisers is of value.

Injection Treatment. Successful control of manganese deficiency in a variety of fruit trees has been achieved by injection of solid manganese sulphate.

Foliage Spraying. Plants can absorb nutrients through their leaves from suitable solutions. Foliage spraying with a solution of manganese sulphate in water is now widely employed for controlling manganese deficiency in fruit and ground crops. The manganese sulphate is sometimes incorporated in routine pesticidal sprays.

Flooding. The flooding of soils, producing conditions in which some of the oxidised manganese is reduced to the available form, is occasionally practised where irrigation is possible. Under certain circumstances, however, the reverse effect has been obtained.

Conclusion. It is not unlikely that the future will see still more reports of manganese deficiency affecting world crops, especially where liming materials are used in the reclamation of soils rich in organic matter, or are applied in excess to any soils. Methods of control of manganese deficiency are being further studied.

INFLUENCES OF MINOR ELEMENTS IN THE MEDICINAL PLANT CULTIVATION : RELATION BETWEEN MINERAL DEFICIENCIES AND THE DISEASES CAUSED BY THE REPEATED CULTIVATION (PRELIMINARY REPORT)

by Koiti Kimura

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a) *Datura.* — It is generally known that there are many plants which are difficult to or can hardly be cultivated repeatedly or continuously in the same soil. We find many such cases in medicinal plants as well as in other vegetables or useful plants. They only keep out of the damages changing the kind of plants in the same places or the different places for those plants which have bad influences each other. The solution of the problem of continuous or repeated cultivation is a matter of great concern for the agriculture, especially in such a narrow country as Japan.

Several hypothesis are seen on such phenomena, for instance : Continuous or repeated cultivation (a) causes self intoxication, or (b) increases the density of bacteria or virus in the soil for plant diseases. Such being the general view, there is no other way but changing plants or places.

(For a) I cannot believe the thought of self intoxication, because in soilless gardening nutrient solution can be used circulatory.

(For b) Following idea came to me : The cause is not only the increase of the density of bacteria or virus in the soil, but the diseases might be caused by the lack of some nutrients, especially some special minor elements in the soil according to repeated or continuous cultivation on the same place. The deficiency of important elements might have weakened the self-defensive power of a plant or put a plant in unhealthy condition.

After a short preliminary water cultivation test with different solutions, deficient in different minor elements, I thought that boron must be most liable to be wasted in the soil. I tried boric acid on *Datura stramonium*, which were planted in the same place for three succeeding years repeatedly and were suffering from unhealthy condition that they could not bear flowers and their leaves were shrinking. 500cc of 0.1 % water solution of boric acid was sprayed on each three plants. Then they began to bear flowers with new ordinal leaves, and after the second spraying a month later they bore fruits.

Encouraged by the result, I am carring out various works to solve the problem.

b) *Digitalis.* — *Digitalis* also can not be cultivated in the same place repeatedly. I have been able to keep *D. purpurea* for 5 years in the same place of my garden, with the solution of minor elements, as a preliminary test.

c) *Ephedra.* — *Ephedra* is very difficult to cultivate successfully in ordinal field. At the experimental field of Tokyo University, they succeeded to keep *E.* growing in good condition at the sea shore. Whether the difficulty is caused by the deficiency of some elements, we are going to carry out the experiment for the problem.

POTASSIUM DEFICIENCY IN RELATION TO PUTRESCINE PRODUCTION

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Potassium deficiency causes soluble nitrogenous substances (e. g. amino-acids, amides, nitrate) to accumulate in plant cells. A study by means of paper chromatography indicates that in deficient barley the two amides are much increased, apparently at the expense of the corresponding free dicarboxylic acids, both of which are diminished. A number of other amino-acids are increased, notably lysine, arginine, glycine, serine, leucine, tyrosine and phenylalanine ; the concentrations of certain others may be almost unaffected : L-alanine, threonine, Y-aminobutyric acid. The amine putrescine is often produced and accumulates until it becomes the most conspicuous feature of the chromatograms, but this occurs only if alkali metals generally are unavailable to the plant.

By supplying putrescine to high potassium barley certain symptoms are induced that otherwise appear only in potassium deficiency, especially characteristic white areas on the leaves, which later die and dry out. Death of whole leaves may follow in a manner simulating potassium deficiency. The internal concentration of artificially administered putrescine that leads to this necrosis in high potassium plants is similar to that of the naturally-occurring substance in deficient plants showing corresponding symptoms. When ample sodium is available to potassium deficient barley putrescine accumulation is slight and this particular necrosis does not usually appear at all. Rubidium completely prevents both the accumulation and the symptom, and lithium apparently does likewise.

High potassium barley metabolizes added putrescine slowly, and the amine disappears from deficient barley in the course of a few days if potassium be supplied ; rubidium and sodium are also effective, but less so than potassium.

Putrescine accumulation is not characteristic of potassium deficiency in plants generally, but all species examined show evidence of disturbance in the metabolism of basic nitrogenous substances. For example, flax may accumulate arginine. Deficient red clover accumulates neither putrescine nor arginine, but instead an unknown ninhydrin-reacting substance which appears to be basic ; the same substance is found in traces in deficient barley. Putrescine administered to high potassium clover is very rapidly metabolized and this same unknown substance appears. It would seem to be toxic, for a marginal and inter-veinal brown necrosis is then induced, the symptoms again closely resembling those seen in potassium deficient clover. Unlike barley, clover has a high diamine oxidase activity ; hence it is probable that under deficiency putrescine is produced in the clover just as in barley, but is immediately converted by the oxidase to the unknown substance.

Feeding experiments have been aimed at elucidating the metabolic route leading to putrescine formation, but this has not been established. It seems likely that arginine is a precursor and accumulates in all species when potassium is lacking, and that the subsequent events depend on the enzyme equipment of the particular plant. The arginine may remain substantially unattacked (flax), or be largely converted to putrescine (barley, wheat, red clover) ; if diamine oxidase activity is high the putrescine is further transformed (red clover). The most obvious route from arginine to putrescine would be via ornithine, which on decarboxylation would yield the amine ; but no evidence of putrescine formation has been found where ornithine has been artificially administered. Moreover, the ornithine taken up disappears only very slowly. Since ornithine is not normally detectable in barley, whether potassium is deficient or not, and since when artificially introduced it is so slowly attacked, it is very unlikely that it can be an intermediate between arginine and the amine. A second possible intermediate is agmatine, another amine produced by direct decarboxylation of arginine. Some evidence has been obtained that administered agmatine may lead to putrescine formation ; moreover a substance that behaves chromatographically like agmatine is often found in traces in potassium deficient barley.

It is not known if potassium is required by any enzyme concerned in normal arginine metabolism, but this appears unlikely ; for if so it must be required in comparatively high concentration, and any other alkali metal would seem able to replace it. An alternative explanation of the phenomena might be sought in the fact that the various substances accumulating in the different deficient plants are all basic, together with the fact that they accumulate only under conditions where very low internal cation to anion ratios, and therefore acid sap, may be expected. Basic metabolic substances might then accumulate in response to the acid conditions, much as organic acids are known to do when cations are present in excess.

A SURVEY OF MINOR ELEMENT DEFICIENCIES IN AFRICA

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In general it may be stated that crop yields in Africa are very much lower than in Europe, or many parts of Northern America. This is true not only of farming carried out by primitive peoples, but also of that by Europeans.

This low yield is not only due to unfavourable climatic conditions. It is found is good years and in areas apparently well suited to the crops produced. Again, the traditional N.P.K. fertilizers do not overcome these poor yields in many areas. The inconsistencies of N.P.K. fertilizer trials show that some other factors must be influencing and controlling growth.

One of the factors which could be of great importance is the presence or absence of minor elements, and it was decided to make a survey of minor element deficiencies in Africa to see how widespread these deficiencies were. As very little has been published on this subject, Government Departments and other large institutions were contacted for information. Information is thus rather patchy and no information is available for some territories. *For the purpose of this survey, all elements other than Nitrogen, Phosphorus and Potassium are considered minor elements.*

It can be stated that minor element deficiencies are widely distributed throughout Africa. They occur on such a scale as to constitute a major agricultural problem. In the Union of South Africa where the most information is available, about 80 % of the agricultural land shows some type of minor element deficiency. The only crop in which no deficiency is known or suspected is sugar cane. At the moment it is impossible to get any idea of the extent of the deficiencies in other territories.

In the Union Zn is deficient in all citrus orchards, as well as in many deciduous fruit districts. As well as Zn, the citrus orchards frequently show deficiencies in B and Cu, and to a lesser degree of Mn and Mg.

Deciduous fruit shows deficiencies of Zn, Mn and Mg while Cereals show deficiencies of Mn and occasionally Cu.

Deficiencies in citrus are very widely reported, firstly because the deficiencies are very widely distributed, and secondly, because these conditions are easily recognised and well known in citrus while in other crops they are not easily recognised.

Lack of S is very much more common than one might suspect. It has been observed as absent in Nyasaland, Southern Tanganyika, Kenya and the Gold Coast. Its absence is readily spotted in tea, although in the Gold Coast the deficiency was observed in groundnuts.

The unsatisfactory nature of the present state of our knowledge is brought out by the fact that no information is available for territories such as Bechuanaland or Somaliland.

Deficiencies that have been observed so far can be briefly summarised as follows for some of the more important areas : 1. *Union of South Africa* (Known deficiencies given in brackets) : Citrus (Zn, B, Cu, Mn, Fe) ; Cereals (Mn, Cu) ; Deciduous fruits (Mn, Mg, Zn, Cu, Fe). — 2. *South West Africa :* Citrus (Fe, Mn) ; Deciduous fruit (Fe, Mn). — 3. *Southern Rhodesia :* Citrus (Zn, Mn, B) ; Deciduous fruit (Zn, Mn, Mg) ; Vegetables (Mo, B, Mg). — 4. *Northern Rhodesia :* Citrus (Zn, Mg, Mn) ; Tobacco (B, Mg). — 5. *Tanganyika :* Citrus (Zn) ; Tomatoes (Mg) ; Tea (S) ; Coffee (Cu) ; Wheat (Mn) ; Cashew nuts (Fe) ; Maize (complex mixture). — 6. *French West Afica :* Groundnuts (Cu, Mg, Mo, B) ; Pineapple (Zn) ; Bananas (Mg) ; Citrus (Zn).

While reporting the wide distribution of minor element deficiencies, it is important to note that they are not all due to deficiencies in the soil. Overliming and other conditions may make these elements unavailable.

Another factor of importance which has not been mentioned is the occurrence of toxic or near-toxic concentrations of some of these minor elements in the soil. Such concentrations have been reported from several areas and can greatly reduce crop yields.

RECHERCHES SUR LA NUTRITION PHOSPHOREE DU BLE

par Victor Caumartin

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En recherchant et en dosant les composés du phosphore, dans chacun des organes du blé, à tous les stades de son développement, nous sommes parvenus à un certain nombre de conclusions nouvelles.

Nous avons constaté que la nutrition minérale phosphorée était ininterrompue. Elle se poursuit jusqu'à la fin de la maturation, au niveau des racines, sans bénéfice d'ailleurs pour le caryopse, puisqu'il n'y a plus alors de migrations possibles. Elle reprend dès le début de la germination, dans le péricarpe humide, par absorption diffuse ; les enveloppes du grain accusent au départ 0,09 % de P^2O^5, 120 heures après 13,71 % et l'enracinement n'interviendra que 210 heures après la mise en terre ; il ne s'agit pas d'un banal phénomène physique puisque ce P^2O^5 se trouve incorporé, par une sorte de métabolisme périphérique, dans des molécules glucidiques (7,18 %), protidiques (5,53 %) et lipidiques (1 %).

Les maxima croissants relevés de la première feuille à l'épi (1re feuille : 0,10 % — 2e feuille : 0,37 % — 3e feuille : 0,41 % — 4e feuille : 0,45 % —

5^e feuille : 0,47 % — 6^e feuille : 0,51 % — 7^e feuille : 0,65 % — 8^e feuille : 0,68 % — épi : 0,95 %) indiquent que la plante ne pouvait atteindre autrement que par paliers successifs le potentiel phosphoré nécessaire à la fructification.

En outre, à aucun moment, même dans les racines, n'apparaissent de formes minérales. Les méthodes d'analyse qui placent les tissus végétaux en milieu acide, même pendant un temps relativement court, détruisent les nucléines au profit de la forme minérale. L'entrée du P^2O^5 dans le circuit du métabolisme, au niveau des membranes des poils absorbants, se fait vraisemblablement par éthérification de glucides.

Les phosphatides jouent un rôle primordial. La perméabilité physiologique est sous leur dépendance ; elles se développent pendant la maturité physiologique du caryopse et leur migration vers le péricarpe est à l'origine du métabolisme périphérique ; c'est grâce à celles apportées par le pollen que l'ovule, malgré son état voisin de la nécrobiose, va poursuivre son développement et utiliser la réserve primaire de glucides phosphorés venue également de la fécondation. Dès qu'elles font défaut, la croissance s'interrompt ; les carences phosphatidiques observées sur la 8^e feuille pendant l'épiaison sont à l'origine de l'arrêt de son développement. Ce sont les phosphatides enfin qui captent les molécules de nucléines pendant la croissance et s'en déchargent pendant la nécrobiose, jouant ainsi le rôle de support de métabolisme.

DEGRADATIONS. RESPIRATION

THE RESPIRATION OF MATURE WHEAT GRAINS IN RELATION TO MOISTURE CONTENT AND FUNGAL CONTAMINATION

by Mary B. Hyde

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The respiration of mature wheat grains has been studied over a range of moisture contents, using a conductometric method to determine the amount of carbon dioxide produced.

The method involves absorbing the CO_2 produced by the wheat in a standard ($N/_{50}$ or $N/_{25}$) solution of sodium hydroxide in a suitable cell, and determining the change in electrical conductivity due to the formation of sodium carbonate. The conductivity is estimated by measuring, by means of dipping platinum electrodes, the resistance of the solution, using a modified Wheatstone bridge circuit. The bridge was judged to be in balance at the point of least sound from an audible signal produced by alternating current from a 1000-cycle R. C. oscillator. By using cells of different sizes, calibrated by dosing with known amounts of carbon dioxide, a wide range of CO_2 production can be covered. Quantities as small as 50 μg. CO_2 can be measured with an accuracy of ± 5 % ; with larger amounts, the accuracy is very much greater.

To measure the respiration, the wheat is placed in a closed container and kept in a water bath at constant temperature. After a given period, the conductivity of the NaOH solution in the attached cell is determined, the air surrounding the wheat is flushed into the cell and the conductivity determined again after absorption of the CO_2 is complete. This process can be repeated several times if required.

In experiments at 25°C, normal wheat (Bersée variety) containing both internal and external micro-organisms was conditioned successively at increasing relative humidities and the respiration measured at each stage. Between moisture contents of 12 and 14 % the respiration rose only slowly (from 0.05 mg. to 0.10 mg. CO_2/100 g. grain/24 hr.), but thereafter there was a progressive increase in the respiration rate. Between 16 and 18 % moisture content the rate rose very rapidly, from 0.50 mg. CO_2 at 16 % to more than 26 mg. at 18 %, and this increase was accompanied by the development of visible external mould.

Similar respiration measurements were carried out on wheat which was free from the internal mycelium reported on at the Seventh Botanical Congress. Development of this mycelium, normally present in grain from countries with temperate climate, was prevented by enclosing the ears a few weeks before harvest in flasks containing bags of silica gel. The air surrounding the maturing grain was thus rendered too dry for the development of fungi. At low moisture contents (below 16 %) such wheat had a respiration rate very similar to that for normal grain. The rate also increased very rapidly at high moisture contents, to almost the same extent as normal wheat, and at moisture contents of 18 % and over was again associated with visible external mould growth.

On the other hand, grain which had been rendered free from both internal and external micro-organisms by spraying the ears during the last few weeks of their development with an aqueous solution of 8-hydroxy quinoline sulphate, and which had a similar rate of respiration up to 15 % moisture, had a much smaller increase in respiration at moisture contents above 16 %, and at 17.5 % moisture, with a respiration of 2.5 mg. CO_2 compared with 25 mg. for normal grain, was still free from external moulds.

Experiments are now in progress on the respiration of grain conditioned simultaneously to a range of moisture contents, and results are not yet complete. It seems, however, from the experiments described above, that the rapid increase in production of CO_2 at moisture contents above 16 % is due largely to the development of external fungi, mainly various species of *Penicillium* and *Aspergillus*. The internal mycelium, consisting principally of *Alternaria* sp. and other genera which produce conidia only sparsely and not at all in the subepidermal position, has relatively little effect on respiration.

MODIFICACIONES ENZIMATICAS DURANTE LA GERMINACION

por Roberto Llamas
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En una serie de investigaciones que aún se prosiguen en el Instituto de Biología de la Universidad Nacional Autónoma de México se han estudiado, sobre todo por Juan Roca y Raúl Ondarza, diversos aspectos de la actividad enzimática durante el proceso de germinación.

El frijol y el maíz, que constituyen elementos muy importantes en la alimentación del pueblo de México, son los vegetales sobre los que preferentemente se han enfocado estos estudios.

En el primero de ellos se investigó la actividad sacarogénica y proteolítica del frijol (*Phaseolus vulgaris*) en sus variedades conocidas como

« negro », « palacio » y « chilar ». La germinación se efectuó en cristalizadores con agua destilada, a temperatura constante entre 20 y 22° C, manteniendo la humedad ambiente a saturación. Los lotes de semillas fueron cuidadosamente pesados y el peso de las que no germinaron se descontó, después de desecación, al peso constante del resto que sí germinó.

Los extractos se hicieron según el procedimiento de Kneen, Miller y Sandstedt pero la estimación de la actividad amilolítica beta y los resultados se expresaron en unidades sacarogénicas y en grados Lintner. El pH óptimo para la actividad sacarogénica fué de 5.4.

La actividad sacarogénica durante el proceso de germinación aumenta progresivamente hasta el décimo día y después desciende en forma irregular.

La actividad sacarogénica se reduce hasta un 50 % cuando la germinación se efectúa en ausencia de luz solar. Dicha actividad es máxima entre 45 y 50° C.

Por lo que respecta a la actividad proteolítica, se utilizó, para demostrarla, el procedimiento de Waldschmidt Leitz y se encontró que el pH óptimo es de 8.0 y que la máxima actividad proteolítica aparece hacia el séptimo día de iniciada la germinación.

Se estudió, además, la presencia y acción de catalasa durante la germinación del frijol, mediante el procedimiento iodométrico de titulación del peróxido de hidrógeno y se estableció que esta actividad es máxima en la variedad frijol negro, menos evidence en el amarillo y menor aún en el blanco.

El estudio de las amilasas alfa y beta en híbridos y variedades de maix mexicano se considera importante porque el maiz hibrido ha sido ampliamente distribuido entre los campesinos mexicanos para la siembra.

La actividad amilásica se expresó en las unidades enunciadas por Kneen y Sandsted o sean :

a) Unidades de alfa amilasa o dextrinogénica. Gramos de almidón soluble que en exceso de beta amilasa son dextrinizadas por un gramo de malta en una hora a 30° C.

b) Unidades sacarogénicas. Gramos de almidón soluble convertidos a maltosa por un gramo de malta en una hora a 30° C.

c) Unidades de beta amilasa. Gramos de almidón soluble convertidos a maltosa por la beta amilasa contenida en un gramo de malta, en una hora a 30° C.

Como conclusiones se expresa lo siguiente :

Tanto la alfa como la beta amilasas del maíz, en las variedades citadas tiene un pH óptimo de 4.6. La actividad amilásica aumenta durante el proceso de germinación y alcanza su máximo a los ocho días. La actividad amilásica es mayor en las muestras aclimatadas para alturas de 2,200 a 2,600 metros y decrece a medida que disminuye la altura de aclimatación. La acti-

vidad proteolítica en variedades de maíz se determina mediante el procedimiento preconizado por Zoch y Olson, y que expresa como unidad los miligramos de nitrógeno liberado en una hora por un gramo de material. Se encontró que la máxima actividad proteolítica en el proceso de germinación de las variedades de maíz estudiadas, aparece entre el quinto y el séptimo días. La actividad proteolítica está en relación con la cantidad de nitrógeno total de las muestras estudiadas. La actividad proteolítica está en relación con la altura de aclimatación.

Por lo que se refiere a la catalasa en variedades de maíz, se utilizó, para valorarla, el procedimiento ya señalado, pero se expresó la actividad en unidades o sea la actividad catalásica que corresponde a la reacción de 1 ml. de peróxido de hidrógeno 0.1N por un gramo de semilla en 60 minutos.

Se encontró que durante el proceso de germinación la actividad catalásica aumenta y alcanza su máximo el séptimo día y que dicha actividad está en relación con la altura de aclimatación.

Résumé. — L'étude des activités amylasique, protéolytique et catalasique des germinations de Haricot et de Maïs a été faite principalement en fonction du temps. L'influence de quelques facteurs (température, pH, altitude d'acclimatation) a été également examinée.

INVESTIGATIONS ON THE PATH OF CARBOHYDRATE BREAKDOWN IN PLANTS

by Harry BEEVERS

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Glucose samples labeled with C^{14} specifically in the 1, 2, 3-4 or 6 position have been used to determine that the direct oxidative pathway or hexose monophosphate shunt (*i. e.* a route distinct from glycolysis) contributes significantly in some tissues to carbohydrate breakdown. In most of the experiments the % yield or respired $C^{14}O_2$ from glucose 1-C^{14} and glucose 6-C^{14} were compared and the fact that in many tissues a higher yield was obtained from glucose-1-C^{14} is regarded as strong evidence for the participation of the oxidative pathway.

HYDROLYSIS OF SUCROSE AND RAFFINOSE AT THE CYTOPLASMIC SURFACES OF PLANT TISSUES

by Husein Saïd

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Evidence is given supporting my contention that when discs from storage organs, cut ends of leaves or cultures of *Fusarium moniliforme* are suspended in aqueous solution of sucrose, the latter is always hydrolysed at the cytoplasmic surfaces of the exposed cells and then the products of hydrolysis (particularly glucose) were subsequently absorbed. The rate of sucrose hydrolysis is always faster than that of sugar uptake and hence the accumulation of reducing sugars. The enzyme responsible for sucrose hydrolysis proved to be fructofuranosidase and not α-glucosidase or sucrose phosphorylase. The fructoside raffinose is also hydrolysed at the cytoplasmic surfaces giving fructose and a disaccharide which is not melibiose, though having the same sugar components.

It seems that melibiose, whether used alone or as a product of raffinose hydrolysis, undergoes rapid rearrangement and becomes transformed into a new disaccharide, still made of glucose and galactose, but gives different properties from all known disaccharide galactosides.

Argument will be raised to show that plant tissues absorb maltose and lactose unhydrolysed, and under certain conditions they can also absorb sucrose unhydrolysed, but when the fructofuranosidase enzyme exists, as it usually does, at the cytoplasmic surface then the sucrose is rapidly hydrolysed and the products of hydrolysis are absorbed more rapidly.

THE SULPHYDRYL GROUPS IN THE RESPIRATION OF THE PLANT CELL

by C. Contreiras and J. E. Baptista

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The respiration (as O_2 uptake) of discs of Ivy leaves, treated with different types of sulphydryl reagents in various molar concentrations, was measured and compared with that of control discs. Under the prevailing experimental conditions it was observed ; (i) that each of the sulphydryl reagents used was able to induce both an increase and a depression in the rate of oxygen consumption of the tissue, according to the strength of the solution applied, (ii) that the occurence of this double effect produced by the sulphydryl reagents seems to be related with the time of contact between reagent and tissue.

The results appear to be in general agreement with those obtained by Barron *et al.* (*J. Gen. Physiol.*, 1948, XXXII, 179) in sea-urchin sperm and therefore support the assumption that also in plant cells, two kinds of sulphydryl groups might be present : the soluble and the fixed groups.

SILICON METABOLISM IN DIATOMS

by Joyce C. Lewin

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Virtually nothing is known of the mechanism by which diatoms absorb silicon from their environment and incorporate it into silica frustules of characteristic patterns.

Investigations on this problem are now being carried out using the freshwater diatom *Navicula pelliculosa* (Bréb.) Hilse. The cells are grown in a mineral medium containing a low concentration of silicon (1 mg./l.). When transferred to a fresh silicate solution (10 mg. Si/l.) and incubated under non-growing conditions, such deficient cells rapidly take up silicon from the medium. This process can be followed quantitatively by colorimetric determinations using the molybdate reaction. (a) It has been shown that the utilization of silicon is an aerobic process. (b) The effect of certain inhibitors on respiration and silicon utilization has been studied. (c) Evidence has been obtained for the participation of sulfhydryl groups at the cell membrane in this process.

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ANALYSE DES PLANTES ET LE PROBLEME DES ENGRAIS

Les communications suivantes seront réunies en un volume spécial. D'autres communications non reçues antérieurement au Congrès, mais présentées au cours des séances, figureront également dans ce volume.

A. **Arland** (*Leipzig*). — Die Transpirationsintensität der Pflanzen als Grundlage bei der Ermittlung optimaler acker- und pflanzenbaulicher Kulturmassnahmen unter besonderer Berücksichtigung von Pflanzenanalysen.

J. **Carles** (*Institut Catholique de Toulouse*). — La Physiologie Normale du Blé.

H. D. **Chapman** (*Department of Soils and Plant Nutrition, University of California, Citrus Experiment Station, Riverside, California, U.S.A.*). — The Analysis of Various Parts of the Citrus Plant as a Guide to Fertilizer Practice.

Bruce J. **Cooil** (*University of Hawaii, College of Agriculture, Honolulu 14, Hawaii*). — Relationships of Leaf Composition to Growth and Yield of the Coffee Tree.

A. S. **Crafts** (*University of California, Department of Botany, Davis, California, U.S.A.*). — Composition of the Sap of Phloem and Xylem and its Relation to Nutrition of the Plant.

M. **Drosdoff** (*U. S. Field Lab. for Tung Investigations, Gainesville, Florida, U.S.A.*). — Role of Leaf Analysis in Fertilization of Tung Trees.

M. **Ferrand** (*I.R.H.O.*). — L'analyse des plantes et la pratique agricole.

J. L. **Haddock** (*Utah State Agricultural College, Logan, Utah, U.S.A.*). — The Interrelationships of Irrigation Method, Soil Moisture Condition and Soil Fertility on the Nitrogen Content of Sugar Beets.

Hallais (*Mauritius Sugar Industry Research Institute, Réduit, Mauritius*). — Nouvelles Techniques de Diagnostic Foliaire au Service du Redressement Alimentaire des Cultures de Canne à Sucre.

Lotte **Herschberg** (*University of California, Citrus Experiment Station, Riverside, California, U.S.A.*). — Leaf Composition in Relation to Nitrogen and Phosphorus Requirements of Citrus Trees in Israel.

W. S. **Iljin** (*Faculty of Agriculture of the Central University, Maracay, Venezuela*). — Yield and Chemical Composition of Commercially-Fertilized Plants in the Tropics.

A. **Loué** (*Centre de Recherches Agronomiques de Bingerville, Côte d'Ivoire*). — Etude de la Nutrition du Caféier par la Méthode du Diagnostic Foliaire.

R. L. Mitchell (*The Macaulay Institute for Soil Research, Department of Spectrochemistry, Craigiebuckler, Aberdeen, G.-B.*). — The Spectrochemical Analysis of Plant Material.

D. J. D. Nicholas (*The Johns Hopkins University, Baltimore, 18, Maryland, U.S.A.*). — Rapid Chemical Tissue Tests for Determining the Mineral Status of Plants.

P. Prévot et M. Ollagnier (*I.R.H.O.*). — Diagnostic foliaire de l'Arachide et du Palmier à huile.

W. Reuther and P. F. Smith (*U.S. Department of Agriculture, Orlando, Florida, U.S.A.*). — Leaf Analysis as a Guide to the Nutritional Status of Orchard Trees.

Roy Overstreet and Louis Jacobson (*University of California, Berkeley 4, California, U.S.A.*). — The Role of Hydrogen Ion in the Mineral Absorption Mechanism of Roots.

A. W. Specht, P. F. Smith and J. W. Resnicky (*Horticultural Crops Research Branch, Plant Industry Station, U.S. Department of Agriculture, Beltsville, Maryland and Orlando, Florida, U.S.A.*). — Simultaneous Spectrographic Procedure and Its Evaluation for Analysis of Plant Materials.

R. M. Samish (*The Agricultural Research Station and Hebrew University Rehovot, Israel*). — A Contribution to the Knowledge of Lime Induced Chlorosis in Grape Vines.

A. F. Steenbjerg (*Department of Soil Fertility and Plant Nutrition, The Royal Veterinary and Agricultural College, Copenhagen, Danemark*). — Manuring and Plant Production.

S. Trocmé (*Station Centrale d'Agronomie, Versailles*). — Quelques Exemples de Contribution de l'Analyse des Plantes au Diagnostic des Insuffisances de Production des Cultures.

T. Wallace (*University of Bristol, Department of Agriculture and Horticulture, Research Station, Long Ashton, Bristol, G.-B.*). — The Application of Trace Elements to Crops.

II. Physiologie de la croissance

1. *CORRELATIONS DE CROISSANCE*

RAPPORT

CORRELATIONS OF GROWTH BY HUMORAL INFLUENCES

LES CORRELATIONS DE CROISSANCE DANS LEURS RAPPORTS AVEC LES PROCESSUS HUMORAUX

by Kenneth V. Thimann

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The first process in which the integration of growth and correlation between parts was traced to a hormone was Apical Dominance. It is therefore appropriate to begin consideration of the many phenomena of correlation with this topic. The simple pattern in which auxin, produced in the terminal bud, inhibits lateral buds lower down was worked out in the 30's. In recent years, however, it has been subject to some modification. Firstly we must note that, as opposed to inhibition, forces tending to promote growth are sometimes controlling. Champagnat in particular (1950 a) has shown how laterals sometimes escape inhibition and form « anticipatory branches », when the growth rate of the stem is at its highest. Conversely Gunckel, Thimann, and Wetmore (1949) found in *Ginkgo* that decapitation did not release the inhibited laterals unless the plants were vigorous ; they concluded that « while the inhibiting influence may be removed, the bud will not necessarily elongate as a result ; its ability to elongate depends on nutritional levels or other factors which. . . can be summarized in the general term ' vigor ' ».

Other instances occur in the behavior of buds in the axils of cotyledons. These have, indeed, been studied extensively by the Czech school, Korinek, Dostal, Komarek and Plch. In many plants, if one cotyledon is removed, the bud in its axil grows faster than that on the opposite side, whose cotyledon is intact. This is the expected hormonal correlation. In *Mercurialis, Linaria* and *Impatiens,* however, it is the bud whose cotyledon is intact that grows the faster, *i. e.* the cotyledon *promotes* the growth of the bud in its axil. *Bidens pilosus* shows dual behavior ; at first the bud growth is somewhat promoted by its cotyledon, but later it becomes inhibited (Champagnat, 1949). Closer examination of this behavior has suggested the explanation : the change over to inhibition occurs sooner, and the inhibition is more complete, in greenhouse plants than in those under artificial light, and correspondingly the former yield the more diffusible auxin from the cotyledons (Champagnat, 1951 a). In other words inhibition is probably due to auxin coming from the cotyledons, but this may not always be enough to overcome the forces, nutritive or otherwise, which promote growth. Also if the terminal apex is removed the inhibiting action of the cotyledons becomes evident, but if only the young leaves in the terminal bud are removed and the meristem remains, some axillary growth occurs and now the cotyledons only promote (Champagnat, 1951 b). One might conclude from this last point that the apex prevents the cotyledons from making auxin. This would coincide exactly with what has earlier been deduced from bud inhibition experiments ; if I may quote my own conclusion (1952) : « There is some reason to think that the inhibition is exerted not so much on the growth of the bud as on its ability to produce auxin. »

An apical dominance of a different type is that seen in *Ginkgo biloba,* where the lateral buds are not completely inhibited, but merely prevented from elongating (Gunckel and Thimann, 1949 ; Gunckel *et al.,* 1949). The resulting « short shoots » produce auxin for a very brief time only, and exert no appreciable inhibiting influence on other buds. If the terminal bud is removed early in the season, at least in young plants, from 1 to 3 laterals will develop into long shoots with many leaves, and greatly prolonged auxin production. Substitution of the terminal bud by naphthalene-acetic acid maintains the laterals in the « short-shoot » form. The transition from short to long shoots is thus clearly controlled by auxin. Yet the shoot and long shoots differ from one another in their auxin production. In these two facts is epitomized the whole problem of bud inhibition.

In other cases the behavior of buds and shoots seems to be less clearly under the direct influence of the auxin supply. In *Cicer arietinum* there are special buds at the base of the stem which are present already in the seed, and which develop without any apparent inhibition if the plant is growing rapidly (Champagnat, 1952 b). Furthermore, lateral buds develop in the middle of the shoots, just where the free auxin content might be expected to be highest (Champagnat, 1952 a). In Lilac the older leaves, whose auxin content shoul be falling off, seem to inhibit bud development more powerfully than the young leaves less than 15 mm. long (Champagnat, 1950). However, the discrepancy in Lilac seems only to be small and certainly does not justify Champagnat's drastic conclusion that « le principe inhibiteur n'est pas l'auxine ». For one must recall that in *Ginkgo,* and very probably in woody shoots in general, the leaves are not the main source of auxin, but the bulk of the diffusible auxin is found in the stem itself (GUNCKEL and THIMANN, 1949). This fact makes it hazardous to try to describe inhibition solely in terms of the action of the terminal bud and the leaves.

A more extensive study, with careful comparisons between auxin content and the inhibition of buds, was made by CAMUS (1949) in tissue cultures of *Cichorium intybus*. CAMUS showed that growing buds inhibit other buds on this tissue, that buds grafted in have the same effect, and that the inhibition is exerted polarly in the direction shoot root. He then determined the auxin content by extracting with ether for 6 hours in the cold, using thin slices of tissue from the apical end, where buds can develop freely, and from the basal end, where they show partial inhibition. The basal end yielded more auxin than the apical end for the first few days, but by the 9th day, when the buds were beginning to develop, and hence the auxin content beginning to increase, the difference became very small. The buds at the basal end, nevertheless, remained only one half to one third as long as those at the apical end. The correlation between auxin level and bud development was thus qualitatively in the right direction, particularly because the first days, when the auxin difference was large, presumably had a great influence on the behavior of the buds which only became visible a few days later.

Nevertheless, because the rate of bud growth was not inversely proportional to the extracted auxin found, CAMUS concluded that « l'auxine ne joue aucun rôle dans les corrélations d'inhibition ». Such a conclusion, which had been formulated previously by PRÉVOT and by GAUTHERET, is totally unwarranted.

We should note several points : (1) while the auxin differences are admitedly small, the bud inhibition is also only partial, (2) there is no *a priori* reason to expect a simple relationship between bud growth and auxin content, (3) we have no assurance that a 6 hour ether extraction measures the auxin available to the budforming tissue. It will be remembered that KRAMER and WENT (1949) could find no relation between the auxin content of tomato plants and their growth, and recently VON ABRAMS (1953) has found in dwarf and normal peas no parallelism between the growth type and either the production or inactivation of auxin. Yet we do not conclude that auxin plays no role in growth. One must not overlook the fundamental experimental fact that pure indole-acetic acid, applied in physiological concentrations, will inhibit buds completely and in a wide variety of plants. Since our original experiments in 1933-34 this fact has been confirmed by many workers ; it has been shown in Prof. GAUTHERET'S own tissue cultures and in the tobacco stem cultures of SKOOG and TSUI (1951). In potatoes the effect is applied on a commercial scale, using the volatile methyl esters of the auxins. One cannot possibly say, therefore, that auxin plays no role in inhibition. It is present, and it must therefore inhibit.

What one can say is that its inhibiting action may be enhanced or reduced by other factors. The influence of general growth vigor was discussed above, but this probably has no bearing on the case of tissue cultures. More important is the supply of other compounds. High phosphate nutrition will overcome the inhibition by auxin. Added adenine specifically promotes the formation of buds (SKOOG and TSUI, 1951 ; STERLING, 1950). Guanine is also effective, and hypoxanthine much less so. Aza-hypoxanthine, an analogue of hypoxanthine, inhibits bud development very actively on the tobacco callus tissue. In presence of adenine, addition of guanine, thymine or cytosine further increases the number of buds formed. In addition to the purines, phosphate and desoxyribose are also effective. Evidently, therefore, bud development depends on the synthesis of nucleic acids. Supporting this, indole-acetic acid concentrations too low to cause appreciable increase in growth cause a 40-50 % increase in both RNA and DNA in 7 days (SKOOG and SILBERGER, unpublished). The nucleic acids are thus a group of naturally occurring materials which exert a « humoral » influence on bud development.

There are other effective substances. Maleic hydrazide, which generally opposes the action of auxin in promoting growth, characteristically breaks apical dominance, causing lateral buds to develop while growth of the main axis is slightly retarded (NAYLOR and DAVIS, 1950 ; BEACH and LEOPOLD, 1953). While admittedly a synthetic chemical, maleic hydrazide may well act in a manner similar to that of naturally occurring auxin antagonists. Evidently, therefore, there is no reason to doubt the fundamental fact that apical dominance is mediated by auxin, but we need only note that there are variants upon the classical theme and there are modifying influences, which are themselves in part of a humoral nature.

The problem of how auxin, a growth-promoting substance, can exert so powerful an inhibition has of course been much discussed in past years and there is little new to add to it. SNOW and others have believed that between the inhibited organ and the auxin there must be at least one intermediate step, an inhibiting substance, if only because inhibition can sometimes be exerted in an upward direction, *i. e.* acropetally. To the older observations on this score may be added three new ones. Removal of lateral buds in *Coleus* stimulates elongation of the terminal bud and growth of its young leaves (JACOBS and BULLWINKEL, 1953). Removal of adult leaves of Lilac accelerates the development of lateral buds higher up on the stem (CHAMPAGNAT, 1951 c). Removal of lateral buds of *Cercidiphyllum* enables the apical shoot, which would otherwise have abscissed, to remain on and to continue to grow (TITMAN, 1953). These supplement the older data in showing that some bud inhibition can occur distal to the source of auxin.

But do we in fact know that auxin transport is always strictly polar ? Acropetal inhibitions are usually far less marked than basipetal ones, so that only a small upward transport of auxin would be needed. Most of the critical experiments, establishing strict polarity, have been done with coleoptiles. Sections of *Phaseolus* hypocotyls transport a definite small amount of the auxin present in them in the acropetal direction (JACOBS, 1950). When auxin is applied externally, however, it is moved polarly if at all (though in very young hypocotyls there is little or no transport in either direction). A small, but definite, amount of acropetal transport took place in stems of *Coleus* (JACOBS, 1952). We know also that acropetal movement can occur in the transpiration stream.

Recently some evidence has appeared that the auxin-transporting system can be readily modified. The studies of ALMEIDA on *Populus* cuttings (1950, 1952) indicate that high humidity is sufficient to stop auxin transport, as shown by bud inhibition, tumor formation and even rooting, at the middle and apical parts of the cuttings. The action of triiodobenzoic acid in preventing tropisms of petioles and in releasing axillary buds from inhibition has been traced by KUSE (1953) to its inhibiting auxin transport. When applied in lanoline below the source of auxin, TIBA partially counteracted the auxin inhibition, or the auxin-controlled tropisms, but when applied above the auxin source it had no effect.

More striking, perhaps, is the demonstration that in flowering shoots of *Coleus* auxin transport below the flower is markedly « depolarized » (LEOPOLD and GUERNSEY, 1954) and that this is probably due to the production by the flower-bud of a diffusible substance modifying transport. Reduced auxin movement in flowering shoots would account for the frequent development of lateral buds at the time of flowering. Experiments on the spraying of tomatoes have been interpreted, also, on the basis of an upward movement of auxin from the main stem into the fruit pedicels (REINDERS-GOUWENTAK and SMEETS, 1953). Admittedly this interpretation is not the only one possible. Such observations show, in any case, that we must not jump to conclusions as to *strictly* polar transport either in growing or in mature shoots, and, if that is the case, then one of the principal pieces of indirect evidence for participation of an apolar inhibitor must lose its value.

Of course there is now plenty of evidence for inhibitors in plants and some of it is very suggestive. Because this subject is treated in another symposium, I shall mention only one or two pertinent cases here. Acid growth inhibitors have been demonstrated by *Avena* tests in many plant tissues, especially by LARSEN and later by HEMBERG in potato and in *Fraxinus* buds (1949). Buds released from inhibition either naturally or by ethylene chlorhydrin yielded less inhibitor, and the same was true for potatoes treated with glutathione (HEMBERG, 1950). Using carrot tissue as assay material, STEWARD and CAPLIN (1952) found potato periderm to yield more inhibitor than the parenchyma, and dormant potatoes to inhibit more than longstored ones. Most suggestive is their obtaining good inhibition from extracts of dormant maple buds, but much less from buds later in the season, while buds swelling in the spring yielded none at all. KEFFORD (1953) in chromatographic studies of auxins and inhibitors in seedling tissues, invariably obtained a strong inhibitor, called β, from lateral inhibited buds, and much less from growing apical buds.

If we agree, therefore, that the substance which travels, and causes inhibition in a predominantly polar (but sometimes apolar) direction, is the auxin itself, it is still possible that the actual process of inhibition is caused by an inhibiting substance. On this view auxin entering the bud would be converted to an inhibitor in the basal tissues of the bud itself (*cf.* the suggestion of BALL (1953) for the action of auxin in rhizomes). It is suggestive, too, that HANCOCK and BARLOW (1953) in chromatographic studies of apple buds and leaves, find that the amount of inhibitor (as measured by growth inhibition of *Avena* coleoptiles) goes parallel with the amount of auxin. Both materials are present in largest amount in the tip and almost absent from mature leaves. We know that isolated buds are inhibited by pure auxin, so we cannot invoke any other tissue as site of the conversion of auxin to an inhibitor. Note that the function of the inhibitor might be primarily to inhibit a process leading to auxin *formation*. Thus all buds, including apical ones, would also form the inhibitor at their base, but since the inhibitor is postulated to act much more on auxin formation than on growth it would be the auxin forming systems, *i.e.* the buds and growing shoots, which would be most sensitive to its action. Release of inhibition would as its first effect allow the auxin-forming enzyme to start functioning.

Recently SKOOG (1953) reported a rapid change in the nuclei of the apex of lateral buds, when inhibition is removed. While inhibited, the nuclei appear vacuolated, and stain weakly ; shortly after the terminal bud is removed they contract and darken. It is tempting to associate such a change with the onset of a new enzymatic activity, namely production either of auxin or of a precursor.

I should like here to mention a problem related to apical dominance which so far has attracted very little attention : why do the buds of woody plants cease growth in the summer, just when external conditions are at their best ? Have they accumulated an inhibitor, or have they exhausted a growth factor ? There is not even a hint of any answer.

Buds of course exert not only inhibiting action, but they also powerfully influence tissue formation. In so far as the activation of cambial divisions and production of xylem is concerned, SNOW and SÖDING demonstrated many years ago that this can be imitated by indole-acetic acid, and JACOBS (1952) has shown that the redifferentiation of xylem strands from parenchyma around a wound is dependent on buds or leaves and can be reproduced by indole-acetic acid. The formation of roots has of course long been established as due to the same cause. The extensive studies on morphogenesis in tissue cultures by GAUTHERET showed that applied indole-acetic acid undergoes polar transport and also characteristically modifies the phloem.

More recently, CAMUS (1949 ; cf. unpublished experiments by WETMORE on lilac) has shown how buds grafted on to tissue cultures of Endive root, or arising spontaneously from them, cause « dedifferentiation » of tissues below, followed by a redifferentiation into vascular bundles ; the conducting elements so formed eventually reach the cambium and stimulate it to divide, forming xylem and phloem in the usual way. All this occurs polarly, *i.e.* in the direction leaf ⟶ root, the direction known to be taken by auxin. Also it can be imitated by indole-acetic and indole-butyric acids.

Two other auxins act similarly but fail to duplicate in detail the action of buds. Furthermore, direct determinations show that the auxin content increases in the tissues exactly when buds appear ; SKOOG and TSUI (1951) have demonstrated the same behavior in cultures of tobacco and of horseradish. BALL's recent study (1952) of *Lupinus* apices isolated from their vascular bundles by surgery shows similarly that the meristem causes formation of a procambium below it which eventually makes contact with the vascular system of the shoot* and matures into xylem and phloem. In any event, we may conclude that the histogenic factor causing differentiation of vascular tissue is auxin, produced by the buds and transported preferentially in the conducting elements.

Before leaving apical dominance some similar phenomena in roots must be mentioned. A clear inhibition exerted by the main meristem and axis on the development of lateral roots has long been known. Root decapitation almost always accelerates lateral root growth. STREET and ROBERTS (1952) have now shown that in tomato roots this is to some extent mutual, *i.e.* that laterals inhibit the growth of the main axis. Red light inhibits growth of laterals (in pea roots) and, perhaps as a result, this too stimulates elongation of the main axis (TORREY, 1952). Very low intensities of white light have a similar effect in tomato (STREET, 1953). In a comparable way, the root nodules of legumes inhibit one another's development (NUTMAN, 1952, 1953). The main root tip also inhibits the development of nodules. It is the meristem of the nodule, and not the bacterial tissue, which is effective. The inhibitors of nodule formation seem also to be secreted by the root into the external medium.

Inhibitions of root growth by different substances have very different morphological and anatomical bases. TORREY (1953) finds that indole-acetic acid and enzyme poisons like iodoacetate or dinitrophenol all inhibit elongation, but the auxin accelerates xylem maturation, while the poisons slightly or completely inhibit it. Thus the auxin, while it inhibits growth, accelerates at least one physiological process, and indeed one which it also

* Wetmore's cultures differ in that the induced procambium remains separate from the shoot vascular system.

accelerates in shoots, while the enzyme poisons, by contrast, appear to inhibit much more generally. Indeed, we have long known that iodoacetate is not a true auxin antagonist. The effects of auxin on growth are paralleled by effects on respiration, but it is hard to tell which is causative (AUDUS and GARRARD, 1953).

Geotropism of roots can be inhibited under conditions were elongation itself is not much affected. This results from treatment with synthetic substances, such as the α and β naphthyl-phthalamic acids (MENTZER and coworkers, 1950 a and b) or 2, 4, 6-trichlorophenoxyacetic acid (BRUMFIELD, 1953) and in consequence has limited significance for natural correlations. It seems probable that these substances specifically affect auxin transport, as suggested above for triiodobenzoic acid in the petiole. The naphthyl-phthalamic acids and related compounds do not affect germination.

Another correlative phenomenon is the abscission of leaves and fruits. This has been well established as due to a *low* level of auxin, and recent work has not challenged this view. SEQUEIRA and STEEVES indeed (1954) have made a valuable application to pathology in showing that leaf spot of coffee, a fungous disease which causes rapid abscission, is almost certainly due to destruction of auxin in the leaf by the fungus. The mycelium certainly destroys auxin actively, and infected leaves yield virtually no auxin by diffusion, while control leaves do.

It has long been known that auxin applied to a petiole in place of a leaf blade will prevent abscission, but ADDICOTT and LYNCH (1951) have found that if the auxin is applied at the proximal end (*i.e.* morphologically below the abscission zone) it actually promotes abscission. This may perhaps be due to some influence on water relations. SHOJI, ADDICOTT and SWETS (1951), however, showed that at the time of abscission the auxin level in the leaf blade falls to that in the petiole and they consider that it is the auxin *gradient*, rather than its absolute level, which determines abscission.

Opposing the action of auxin, *i.e.* promoting abscission, in cotton are many synthetic compounds like Endothal and ethylene chlorhydrin ; trans-cinnamic acid has a small but real effect, and ethylene gas is very effective (HALL, 1951-52). Since cotton leaves do produce ethylene, the control of abscission has been ascribed by HALL to a balance between ethylene and auxin. The young leaves unfortunately produce more ethylene than the old, but of course they do product more auxin also. The fact that, in *Coleus*, leaves promote abscission of other petioles near them has been ascribed by ROSSETTER and JACOBS (1953) also to traces of ethylene formed by the leaves, though the evidence for this has not yet been given.

Our last topic is humoral correlations in reproduction. Though most workers continue to talk and write about « Florigen », no evidence for such a flower-forming hormone is yet to hand, and we must tentatively conclude that it does not exist. Indeed, the whole Sachsian idea of specific *organ-forming* hormones is too superficial to be retained in modern physiology. The formation of organs requires a complex interplay of factors, not just a single one. It must depend on the *relative rates* of a number of processes, and on the supply, removal and destruction of many factors. In flower formation there is a great deal of evidence that one of these factors is auxin. Many earlier experiments indicated that auxin treatment antagonizes flowering, and perhaps some of these effects were due merely to inhibition of the development of buds and therefore to inhibition of their conversion from vegetative to flower primordia.

Xanthium canadense, a short-day plant, is completely prevented from flowering on short days if sprayed with auxin or if auxin is applied to the base of cuttings. The auxin-treated leaves also do not transfer the flowering stimulus when grafted on to other plants (Bonner and Thurlow, 1949). Auxin also prevents flowering of the monocotyledons *Hordeum* (long-day) and Teosinte (short-day) (Leopold and Thimann, 1949). It also delays flowering in the long-day plant *Calendula* (von Denffer and Gründler, 1950). Other examples are summarized by Lang (1952). Also yeast extract, which contains auxin, delays flowering of peas (Haupt, 1952). X-radiation, which destroys auxin, slightly but definitely increases the number of flower primordia of barley (Leopold and Thimann, 1949). Ultraviolet hastened flowering of two long-day plants, *Statice* and *Linum*, but others were unaffected (von Denffer and Schlitt, 1951). Also triiodobenzic acid (Galston, 1947 ; Bonner and Thurlow, 1949 ; Claes, 1952) and coumarin (Leopold and Thimann, 1949) which oppose auxin action, increase flowering in various plants.

A particularly clear inhibition of flowering by auxin was reported by Harder at the last Congress, with *Kalanchoe* plants reduced to two leaves. If one leaf is placed in short day and the other in darkness the plant flowers, but if auxin is applied to the darkened leaf, flowering is prevented (van Senden, 1951). Unlike triiodobenzoic acid and coumarin, maleic hydrazide, which also opposes the action of auxin, does not hasten flowering, but delays it in many plants (Naylor and Davis, 1950). Indeed it has been applied in horticulture for this purpose (White and Kennard, 1950), but this action, like that of high auxin concentrations, probably depends on a more general inhibition of bud growth (Klein and Leopold, 1953). However, in the pineapple, auxin was long ago shown actually to induce flowering, and in barley also low auxin concentrations significantly promote or hasten flowering (Leopold and Thimann, 1949).

Whether auxin promotes or inhibits flowering seems to depend to some extent on temperature. In peas, barley and soybeans, flowering is delayed to a later node or the number of flowers formed is decreased, if the auxin treatment is followed by temperatures of 18-20°. Flowering is advanced, or the number of flowers increased, when the same auxin treatment is followed by incubation at 3° or 10° (Leopold and Guernsey, 1953, 1954). Some of the variation in results of treating seeds with auxins, reported formerly, may be due to this cause. Thus auxin can under some circumstances, and in several plants, promote flowering.

Also « auxin antagonists » cannot in general produce flowering, beyond perhaps a very small effect at the extreme threshold of photoinduction where « flowerlike buds » were reported formed (BONNER, 1949). These facts make improbable the generalization that flowering, like abscission, simply results from law auxin levels (LANG, 1952). It is true that in flowering shoots the auxin production very often decreases, as shown by stopping of cambial divisions in the flower-stalk (STRUCKMEYER, 1950). In clover the auxin level in young flower buds is reported to be very low, and it increases with development, especially in the pollen (ALEKSEEV and STARTSEVA, 1950). There are exceptions, though, as in the case of *Populus*, where the male catkins do not stimulate the cambium below them, while the female ones do (REINDERS-GOUWENTAK and VAN DER VEEN, 1953).

Many years ago UYLDERT showed the flower-stalks of daisies (*Bellis perennis*) to produce auxin, and similar observations have been made since on various plants, especially by SÖDING. More striking are the plants with rapidly-growing flower-scapes ; VON DENFFER (1952) points out that *Agave* flower-stalks « shoot » at a phenomenal rate, so that their auxin production must be at a maximal, not a minimal, level. We conclude, then, that the auxin level is not itself the causative agent in flowering, though it may modify, and often inhibit, a flowering response induced by other factors.

Yet flowering is under a *humoral* influence, since the effect of day-length, perceived by leaves, causes changes from the vegetative to the flowering state in distant buds. The humoral influence is stable, since isolated leaves, exposed to short-day, can cause flowering when afterwards grafted on to a non-induced plant (LONA, 1949). Ten days after the last inductive photoperiod *Xanthium* leaves can still transfer the flowering stimulus when grafted to a host plant (BONNER and THURLOW, 1949). It is the formation of this humoral material which is apparently prevented by auxin. Studies of leaf metabolism (which I shall report in another section) suggest that this humoral material may be an enzyme or a complex of enzymes (GREGORY *et al.*, 1954 ; SPEAR and THIMANN, 1954).

Other correlations in post-floration phenomena are well established as due primarily to auxin. Because these will be discussed in another section, they can be dealt with relatively briefly. Suffice it to say that in addition to the auxin supplied by the pollen tube, the bulk of the auxin needed for fruit growth appears to be formed by the developing seed. The best evidence for this is seen in the strawberry (NITSCH., 1949, 1950, 1952). In this fruit, removal of the « seeds » (or achenes) which are on the outside stops growth of the « fruit » (*i.e.* the receptacle) almost at once. If one or two achenes only are fertilized, each becomes surrounded with a small mass of developed fleshy tissue. Synthetic auxins could fully replace the action of the seeds, just as shown for so many other fruits by GUSTAFSON and others.

The biochemical basis for the action of fertilized ovules, or of auxin excreted by them, in causing ovary growth has as yet been little analyzed. Major changes in carbohydrate metabolism appear to be involved. The ovaries and receptacles of orchids show marked increases in sugar content, especially in starch, after pollination (HSIANG, 1951), and starch is also rapidly synthesized in the fruits of *Erodium, Pelargonium* and tomato (MARRÈ, 1949 ; MARRÈ and MURNEEK, 1953). Not only do the starch and reducing sugars increase, at the expense of sucrose, but the total carbohydrate content of the fruit increases greatly, that is, it accumulates carbohydrate from other parts of the plant (MARRÈ and MURNEEK, 1953). These effects are all duplicated by synthetic auxins ; 4 chlorophenoxyacetic acid raised the soluble sugar content of tomato ovaries to 2-1/2 times that in the leaves. Growth of the ovary in response to auxin is promoted by malic acid, glutathione, manganese salts, and ascorbate, and is inhibited by CO_2 and pyruvate. This suggests that the decarboxylation of malic acid may be involved in the process (LEOPOLD and GUERNSEY, 1952). In *Pelargonium*, emasculation of the flowers causes the sucrose content to fall by 50 or 75 %, while the reducing sugars rise, and indole-acetic acid in lanoline brings the values back to normal (MARRÈ, 1951). This calls attention to the stamens as the first center of auxin production.

Just how, and from what, the fertilized ovule produces auxin, is far from clear. Most of the auxin is in the endosperm. The maximum auxin content of the apple seed coincides with the completion of the digestion of the nucellar tissues by the endosperm (LUCKWILL, 1948). This, in turn, about coincides with a maximum in the free (non-protein) tryptophane content of the strawberry seeds (NITSCH, 1952). However, the receptacle, which produces no auxin, contains more free tryptophane than the seeds. Thus if tryptophane is indeed the precursor of indole-acetic acid, it is not the limiting factor. Probably it is the enzyme system for conversion which plays the limiting role. It may be noted in passing that the reaction tryptophane ⟶ indoleacetic acid would probably go via indole-pyruvic acid, and the recent finding of this acid in corn (STOWE and THIMANN, 1953) strengthens the likelihood that this is the path of biogenesis of auxin.

As a last point, we may note that, while the seed influences the growth of the surrounding tissue, its own growth is no less influenced, at least in its earliest stages, by that peculiar tissue the endosperm. Very young embryos which will not develop in artificial media are enabled to grow by the addition of extracts of endosperm ; — corn, malt and especially coconut milk. Despite vigorous investigation by three groups of workers, at Wisconsin, Cornell, and the Lederle Laboratories respectively, the chemical nature of these endosperm factors remain unclear. They are evidently multiple, and at least part of their effect can be produced by amino-acid mixtures ; part also may be due to the content of auxin, potassium and other well-known materials. Thus we do not know whether a really new growth factor is concerned or not.

It will be evident from this brief survey that hormones act *in* or *on* every organ of the plant, and at every stage of development from the cradle to the grave. The plant, not endowed with a nervous system, yet achieves a mutual influence of its parts upon one another which enables us to think of it as an integrated whole. And one must remember too that humoral influences are not only the internal messengers of correlation, they are also the means whereby the plant responds to external stimuli. We have long known how gravity and light act via the auxin system ; we can see now many ways in which temperature and humidity can do the same ; photoperiodism too acts upon a humoral, if not a hormonal, mechanism. Many years ago Sir Charles Sherrington, the neuro-physiologist, laid primary stress upon the *integrative* action of the nervous system in animals ; correspondingly one may suggest that botanists might well become accustomed to thinking of the integrative action of the humoral system in plants.

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AUXIN AND ETHYLENE IN THE REGULATION OF ABSCISSION

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This paper is a review of current ideas on the regulation of abscission, and includes a discussion of pertinent experiments, an evaluation of the current hypothesis, and the presentation of a new hypothesis.

Since the work of Laibach, 1933, it has been recognized that auxin can retard abscission. Laibach's results have been repeatedly confirmed and have led to the development of important agricultural uses of auxin-like substances to retard abscission. About the time of Laibach's work it was also recognized that ethylene can accelerate abscission, and in 1943 it was found that calcium cyanamid can accelerate the leaf abscission of cotton. These results led to important agricultural uses of ethylene and calcium cyanamid, and of a variety of other substances, such as magnesium chlorate, sodium ethyl xanthate, and sodium endoxohexahydrophthalate, to accelerate abscission. Due to the effectiveness of ethylene at very low concentrations (often one part per million, or less), and to its ability to stimulate a wide variety of other plant responses, interest in it has continued.

In 1942 Milbrath and Hartman showed that auxin and ethylene are mutually antagonistic in their action on abscission, and by pretreatment with napthaleneacetic acid prevented the ethylene-induced shed of holly leaves and berries in transit. In 1950 Gawadi and Avery proposed a hormone-ethylene hypothesis of the regulation of abscission, a hypothesis actively supported by W. C. Hall, 1952. This hypothesis postulates the control of abscission by the relative amounts of ethylene and auxin present in the organ being abscised : if its ethylene is relatively high, abscission occurs ; if its auxin is relatively high, abscission does not occur. This hypothesis is attractive, and a number of observations can be interpreted as supporting it. But it has serious defects :

(1) A number of substances, including oxygen and phenyl mercuric oleate, accelerate abscission.

(2) There are several instance of acceleration of abscission by application of auxin and auxin-like substances.

(3) There is as yet no evidence of the functioning of ethylene in the field. Its high volatility and the failure of repeated efforts in the field to accelerate abscission by its application suggest that it does not function there.

(4) The hypothesis focuses attention on the organ being abscised rather than on the subtending abscission zone, which is the site of action leading to abscission.

There is no doubt that auxin and ethylene are mutually antagonistic in their effect on abscission. Data on this point will be presented. What is doubted is that ethylene is an intrinsic, endogenous regulator of abscission.

Another hypothesis is here presented, an auxin gradient hypothesis. This hypothesis postulates the control of abscission by the auxin gradient across the abscission zone : Abscission does not occur when the auxin gradient, as is usual, is high in the distal portion and low in the proximal portion of the abscission zone. Abscission occurs when the gradient becomes low or is reversed. This regulation prevails under widely varying conditions ; it fails only when the auxin concentration on the distal side is extremely high ; then abscission does not occur even with a reversed gradient.

This hypothesis was suggested by the recent work of ADDICOTT and LYNCH showing that while distally applied IA (indoleacetic acid) retarded abcission, proximally applied IA accelerated abscission. The hypothesis is supported by the known facts. The accelerants of abscission can all be interpreted as lowering or reversing the usual auxin gradient across the abscission zone ; the retardants and inhibitors of abscission can all be interpreted as maintaining or raising the gradient. The argument in support of this hypothesis will be presented in detail.

It is hoped that this hypothesis will form a basis for work on the biochemical effects of accelerants and retardants of abscission leading to a more complete understanding of the regulation of abscission.

GROWTH SUBSTANCES AND GEOTROPISM OF ROOTS

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Geotropism is commonly regarded as a growth response, due to an asymmetric distribution of auxin (the Went-Cholodny theory). CHOLODNY propounded for roots, that a growth substance is produced by the root tip. This substance has a retarding effect upon the growth rate of the root and is removed by gravity in such a way that it is accumulated in the lower part of a horizontally placed root. As a result of this redistribution, growth is retarded on the lower side of the root and a curvature is originated. The fact that auxin inhibits the growth of roots is explained by the hypothesis that roots contain superoptimal concs. of the substance.

If this is true, it can be postulated, that an increase or a decrease of the auxin conc. in a root will affect the geotropic response. The alteration of the auxin contents can be evoked by treating the roots with auxin or an auxin antagonist. In my experiments the roots were submerged in water solutions of β-indole-acetic acid and two substances that in respect to the growth of wheat roots have an antagonistic effect to this substance. It could be shown, that the geotropic response of roots is a combination of the normal positive reaction and a weaker counter-reaction in the opposite direction. This counter-reaction is normally covered by the former and has a longer presentation time than this reaction has. It coult be shown too that β-indole-acetic acid has an effect upon the positive reaction, appearing as a prolongation of its presentation time and reaction time. When the positive reaction was retarded by the β-indole-acetic acid the counter-reaction came into appearance. The latter is affected by α-p-chloro-isobutyric acid, which on the other hand has no effect upon the positive reaction. It was therefore assumed that the two substances mentioned are not real antagonists.

Another substances, α-3-indole-isobutyric acid, that also promotes the growth of roots, is used for the experiments. This substances seems to have an effect upon the geotropical response, not identical with that of α-p-chloro-phenoxy-isobutyric acid. Thus it is able to change the normal positive geotropic response to a negative one. The roots are bending upwards. A similar effect is not called forth by α-p-chloro-phenoxy-isobutyric acid. The substance may be a true antagonist to the β-indole-acetic acid.

These experiments are just in work, and therefore a true text of my paper can not yet be presented.

GROWTH-REGULATORS AND NUTRITIONAL RELATIONS IN THE PLANT AND THEIR CONNECTION WITH DEVELOPMENT

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Many data have been elaborated, especially in the past, in order to clear up relations between nutrition and development under different aspects, calling attention at times to the trophic gradients of the plant.

There followed later on, the period of research on hypothetical florigenous and antiflorigenous hormones, until we reached the present question of auxine/antiauxine balance in relation to development. The studies of auxine and antiauxine action on plants under subcritical conditions for flowering, have reached at times apparent qualitative and specific results on development (auxines generally manifesting an antiflorigenous capacity while the antiauxines — on the contrary — exibited an euflorigenous one) ;

very often results are evidently only of a quantitative order. Similar results are on the whole very analogous to those obtained, in preceding times, by dosing (limitation or, on the contrary, perfusion) of nitrogen in mineral nutrition ; also in similar circumstances, effects at times qualitative and specific, at others (and more often) only quantitative, have been obtained (1).

Some data indicate that plants, for example Soya Bean, most sensible to auxine and antiauxine tratment, are also more sensible to dosing of nitrogen nutrition.

On the other hand, it has been observed that there are perceptible relations between phytoregulators and nutrition (Avery *et al.* demonstrated that a high nitrogen nutrition leads to a relative hyperauxinia).

In agreement with Thimann's hypothesis (which establishes a relation between auxine action on flowering and the hypothesis of Clebs on C/N action (2)) it now becomes necessary to substantiate with new data the statements above mentioned.

The importance of these facts may also increase, if certain data are confirmed, which, on the other hand, seem to attribute to auxines and antiauxines modifying capacity of trophism and trophic gradients.

Although these facts do not directly affect the qualitative question of flowering mechanism, they may interest the present debate for a more complete interpretation of experiments on development.

Putting aside for the moment the question of trophic gradients and referring, that is, to the plant « in toto », let us consider first of all that the auxines in general tend to keep plants young (while some antiauxines tend to carry them towards senescence). On the other hand, let us consider that a low rate of C/N (Tables) corresponds to a juvenile state of the plant. If it were possible to demonstrate an indispensable mutual-dependance between these two facts, we could affirm that the auxines, in order to rejuvenate the plant (or better still to prevent ageing), must be able to lower the C/N ratio.

Therefore we might consider auxine action on development, above all as a quantitative action on anthogenous maturity, which have different degrees (Lona, 1950) (Diagrams) (or an action only on anthogenous maturity and therefore quantitative). The same role may be attributed to C/N ratio. Clebs himself has simply attributed a responsability to C/N only in regard to « Blühreif Zustand » of the plant and not directly on floral initiation.

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(1) We consider here only nitrogen without forgetting the role of other elements which reflects this argument but which for the moment are not discussed.

(2) Relation C/N which is fit for discussion both as nature of two groups of substances — carbohydrate and nitrogen compounds — and as action in relation to the extension of ratio.

All this does not preclude the possibility, completely supported by myself, that those material conditions which stimulate or inhibit (by anthogenous induction and by absence of induction, respectively) flowering in photosensible plants which have reached a more or less elevated degree of anthogenous maturity, may be identifiable with specific substances, possible of hormonic nature. How then a plant in a less mature state, may bloom by induction more quickly than a more mature one under absence of induction, would not be difficult to understand, if we only gave to the several stages of maturity the faculty of permitting a more or less easy induction.

Particular treatments with auxins and nitrogen compounds have a delaying effect on flowering on account of their « antiripening » action on the plant ; they increase their photoperiodic exigences as if they decreased their age.

The second point I should like to consider (regarding interaction between auxines and nutrition on development) consists particular phenomena of trophic correlations and nutritive gradients which take place more or less evidently in many plants following auxine treatments. Also in regarding to this point and especially in regard to it, I want to make clear that those are phenomena which can have only a quantitative role in flowering ; I want to underline however their importance in the interpretation of the experiences on development.

These correlative phenomena include effect of auxines on translocation of materials and their utilization. Like I myself could demonstrate, auxines in large doses can hinder in some plants transport of material from leaves to stem and buds, which can produce a delay in flowering, when leaves under anthothrope conditions are treated. Another exemple of interference in flowering due to correlative factors has been recently studied by myself in shortday suffrutex *Salvia longistyla,* which, treated with IAA, very evidently delayed flowering through a phenomena strictly correlated with induction by IAA of a mass migration downwards of elaborates (with utilization in the formation of adventitious roots and also — a reaction, this, particular to *S. longistyla* — with growth of basal sprouts) so that we observed a phenomena of a starving-out of the apical parts, where flowering normally occurs. At high concentrations there is not flowering whatever of these parts, while a slow vegetative organogenesis can still be seen with formation of very small leaves in an even greater number than the controls form before flowering. Such vegetative organogenesis must take place not by specific processes but by the fact that the level of necessary flowering substances does not reach the optimal quantity, as takes place in plant buds, which, also being under euflorigenous photoperiodic conditions, suffer from a severe limitation in nutrition.

The result of many experiments on the action of IAA in report to flowering must be controlled when these correlative phenomena interfere, and

especially when contemporany phenomena of utilization of trophic materials take place in the formation of adventitious roots — or also of root primordia only — and of other organs to the detriment of bud nutrition.

CONTRIBUTION A L'ETUDE DU ROLE DE L'AUXINE SUR LA RHIZOGENESE

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Nous avons voulu inhiber, sans l'utilisation de composés chimiques, la propriété rhizogène des fragments des tiges qui s'enracinent normalement, puis annuler cette inhibition par l'auxine. Il s'agissait d'établir ensuite, et ceci constituait le point essentiel de cette étude, si des tissus péricycliques isolés ou accompagnés de ceux situés au-delà du cambium et traités comme les fragments de tiges réagissaient de la même façon.

Prélever aseptiquement les organes et tissus sans provoquer de lésions sur leurs cellules épidermiques et corticales constituait la difficulté majeure de cette expérimentation ; en utilisant les tiges d'*Aucuba japonica* et une technique personnelle, il nous a été possible de l'écarter. La preuve en est que même les cellules épidermiques des explantats prolifèrent.

Voici, schématisés, les résultats fournis par des cultures parallèles.

A. — *Cultures dans le milieu de base sans auxine (Knop 1/2 +glucose + gélose).*

Série A : des boutures d'*A. japonica,* longues de 3 cm, pourvues d'un bourgeon, placées dans ce milieu, s'enracinent. Des fragments d'entrenœuds (3 cm) sans bourgeon forment un cal et ne donnent ni ébauches ni racines. Ces faits confirment le rôle du bourgeon dans la néoformation des racines, si élégamment étudiée par Camus (1949) et ses devanciers.

Série B : devant l'impossibilité d'isoler et de traiter les tissus péricycliques seuls, des lambeaux larges de 5 mm et longs de 3 cm, comprenant le tissu cortical et libérien, sont cultivés. Ces tissus prolifèrent sans donner non plus de racines.

B. — *Cultures dans le milieu de base contenant de l'acide indol-* β*-acétique (1 à 100 mg/1).*

Nous n'insisterons que sur deux cas : fragments d'entrenœuds et lambeaux. La superposition de l'action du bourgeon et de l'auxine sur la rhizogénèse nous éloignerait, pour le moment, du but.

Série C : des fragments d'entrenœuds identiques aux précédents donnent des racines. Leur nombre croît avec la dose d'auxine en même temps que leur apparition polarisée diminue. On ne peut pas cependant établir avec certitude une relation directe entre nombre de racines et doses d'auxine.

De plus, le volume du cal diminue au fur et à mesure que la dose d'auxine croît ; en présence de doses élevées d'auxine, il ne se forme que des racines. Il existerait donc dans ce cas un antagonisme marqué et probablement indirectement contrôlable par l'auxine entre des cellules néoformées se plaçant d'une manière archaïque et celles qui s'orienteraient selon un plan défini pour donner des racines. L'ensemble de ces faits ne paraît pas être en accord avec l'hypothèse d'après laquelle l'auxine favoriserait la rhizogénèse en tant que facteur de la division cellulaire. Ils doivent cependant venir à l'esprit, pensons-nous, lorsque nous attachons peu de crédit au concept hormonal.

Série D : des lambeaux comparables à ceux de la série B prolifèrent un peu mieux que ces derniers. Mais en opposition avec ce qui se passe sur les fragments d'entrenœuds de la série C, il n'y a pratiquement ni formation ni ébauche de racines. Sur 110 cultures, une racine est apparue. Un simple calcul montre que si les lambeaux n'étaient pas séparés des fragments d'entrenœuds, ils auraient fourni plus de 400 racines et ébauches de racines.

Malgré la netteté des résultats, l'interprétation correcte des faits reste néanmoins difficile. L'origine péricyclique des racines de *A. japonica* nous a paru certaine. Néanmoins, quelques indices fournis par l'unique explantat enraciné dans la série D nous font penser à la possibilité d'une action à distance des cellules cambiales.

La question fondamentale est de savoir quel est le facteur qui manque, en partie ou en totalité, dans les lambeaux de la série D. Est-il un composant du complexe rhizocalinique, et dans ce cas, lequel ? Est-ce une véritable hormone déterminant seule la rhizogénèse et qui serait formée ou plutôt libérée, en présence de l'auxine dans les tissus de la tige associés aux lambeaux avant leur prélèvement ? Ou enfin, s'agit-il d'un métabolite banal de la nutrition, lequel, en présence de l'auxine et de l'ensemble des tissus des fragments de tiges, permettrait l'établissement d'un équilibre nutritif approprié à l'apparition des racines ?

L'ensemble de ces expériences semble prouver que l'auxine n'agit pas sur la rhizogénèse en tant que biocatalyseur général de la croissance, ni seule, ni après être combinée avec un ou plusieurs facteurs toujours présent dans les cellules péricycliques. L'intervention d'un facteur mobile vient immédiatement à l'esprit.

Nous pensons donc que, tout en attribuant un rôle prépondérant à l'interdépendance des réactions métaboliques de la croissance, la néoformation des racines serait difficilement explicable si nous rejetons le concept hormonal. Le rôle spectaculaire du bourgeon et de l'auxine sur *l'enracinement des fragments de tiges inaptes à donner des racines* (séries A et C) *et l'inefficacité de l'auxine sur les tissus péricycliques isolés* (série D), *cultivés sous des conditions bien définies,* plaide nettement en faveur d'un tel processus.

RECHERCHES SUR LA GERMINATION DES GRAINES DE L'OROBANCHE PARASITE DU TABAC (*PHELIPOEA RAMOSA* C. A. MEY)

par Camille **Izard** et Henri **Hitier**

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L'Orobanche rameuse qui occasionne parfois des dégâts importants dans les cultures de tabac, a fait l'objet de recherches à l'Institut Expérimental de Bergerac, recherches qui ont abouti à des conclusions nouvelles tant du point de vue biologique que du point de vue pratique.

Tout d'abord, nous avons essayé de faire germer les graines d'Orobanche en présence de jeunes racines de tabac. Dans ce but, on disposa un tampon de coton, imprégné d'eau distillée, dans un tube à essais, en ayant soin de saupoudrer de graines d'Orobanche la partie inférieure du tampon, alors que la partie supérieure recevait quelques graines de tabac ; celles-ci germèrent sans que leurs racines atteignent les graines d'Orobanche. Après une vingtaine de jours à l'étuve réglée à 22°C, on observa une graine d'Orobanche nettement germée. On pouvait donc conclure que le contact avec une racine de tabac n'était pas nécessaire à la germination des graines du parasite. D'ailleurs, le fait devait être confirmé par une expérience ultérieure où nous avons obtenu quelques germinations, en utilisant simplement de l'eau distillée, dans laquelle nous avions mis des graines de tabac à germer.

Après ce premier résultat, de nombreux essais ont été effectués en mettant les graines d'Orobanche en présence de sucres et de vitamine B_1, mais aucun succès ne fut obtenu. Nous avons alors cherché à provoquer la germination des semences de *P. ramosa* en utilisant des substances chimiquement définies susceptibles d'être élaborées par les racines de l'hôte. Nous avons tout d'abord expérimenté avec la nicotine et la nicotinamide (vitamine PP). Dans les essais, les graines d'Orobanche étaient mises sur papier filtre alimenté par les solutions actives et maintenues à 24-25°C. La nicotine-base comme la nicotinamide furent utilisées en solutions aqueuses à raison de 1, 10, 50 et 100 mg au litre. La nicotine n'a provoqué aucune germination dans les conditions de l'expérience. Par contre, nous avons obtenu jusqu'à 60 % de graines germées, après 34 jours de culture, avec la vitamine PP utilisée à la concentration de 10 mg au litre. La vitamine PP provoquant la germination des graines de l'Orobanche du tabac, nous avons essayé la vitamine B_6 (pyridoxine), qui possède également le noyau pyridine. Bien que moins active que la nicotinamide, la vitamine B_6 provoqua également un certain nombre de germinations (8,3 % à la concentration de 10 mg au litre). C'était la première fois, à notre connaissance, que l'on obtenait la germination des semences d'une Orobanchée à l'aide de substances chimiques définies et douées d'un rôle vitaminique. Ainsi, en ce qui concerne la spécificité biochimique des substances propres à lever l'inhibition des embryons de *P. ramosa*, il semble établi que le noyau pyridine soit indispensable ; cependant, sa présence ne suffit pas pour déterminer la germination (cas de la nicotine). Il est nécessaire que la substance soit également douée d'un rôle vitaminique ; l'amide de l'acide nicotinique est précisément une de ces substances et l'on sait qu'elle intervient plus particulièrement dans l'oxydation des sucres et de leurs esters phosphoriques.

Les résultats obtenus par Grooshevoy à l'Institut Soviétique du Tabac qui préconise une culture de luzerne pour éliminer l'Orobanche rameuse d'un champ infesté s'expliqueraient ainsi par le fait qu'un grand nombre de légumineuses secrètent au cours de leur croissance une forte quantité d'acide nicotinique. Effectivement, nous avons obtenu la germination de quelques graines de *P. ramosa* en les mettant en présence de racines de luzerne cultivée en pot. Un certain nombre d'essais ont été entrepris et certains d'entre eux sont en cours, pour préciser le mode d'action de cette légumineuse. Nous avons cependant constaté que les extraits de racines riches en nodosités sont susceptibles de provoquer la germination des graines du parasite, alors que nous n'avons obtenu aucun résultat en utilisant des extraits radiculaires de plantules de luzerne.

Par ailleurs, il est possible que la luzerne ne soit pas la seule plante non parasitée par *P. ramosa* et cependant capable d'en faire germer les graines. Nous avons en effet obtenu un très faible pourcentage de germination des graines de *P. ramosa* sous l'action d'extraits de semences d'avoine. Des expériences seront néanmoins nécessaires pour préciser dans quelle mesure de telles plantes pourraient être utilisées pour lutter contre l'Orobanche du tabac.

2. *CROISSANCE NORMALE ET PATHOLOGIQUE*

a) *MECANISME DE L'ACTION DES AUXINES*

RAPPORT

THE MECHANISM OF AUXIN ACTION

by L. J. Audus

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INTRODUCTION

In recent years thousands of compounds active in controlling the growth and development of plants have been discovered. It is necessary therefore first to decide what we mean by an auxin. Fortunately Thimann has given us an unequivocal definition which restricts auxins to substances active in promoting the extension growth of plant cells. However auxins usually modify other phases of the plant's growth and development and it would be very shortsighted to neglect these other aspects in our deliberations. Indeed this very multiplicity of plant response caused Thimann to postulate his « master reaction » — a physiological or biochemical process fundamental to the functioning of any growth system and one in which auxin is an essential component. This theory has been widely accepted and forms today the background of most researches on the subject.

Before we proceed we should consider the identity of the natural auxins. Thus it has been suggested that auxins A and B, as characterized by Kögl, are the only natural auxins and that β-indolylacetic acid and its synthetic homologues owe their actions in growth to an « activation » of one or both of these substances. Obviously such an attitude must materially modify theories based on the responses of plant tissues to external applications of synthetic substances. I do not subscribe to this view and am persuaded, from the rapidly accumulating data of chromatographic investigations, that β-indolylacetic and some of its close relatives are true natural auxins and also probably the most important ones. The very existence of auxins A and B is very much in doubt. We must therefore, in the light of such knowledge, include under auxins all compounds that evoke the true auxin response when applied to test organs. There still remains however the ambiguity of those compounds that do not evoke such a response but which have actions resembling those of auxins in other growth phenomena. On these substances we can at the moment do nothing more than keep a watchful eye and an open mind.

Work on the mechanism of auxin action has fallen roughly into four main categories. These will now be considered separately.

Auxins and the water relations of the extending cell.

The conditions necessary for water absorption by any cell is that the suction force exerted by that cell shall exceed that of its environment. For a cell therefore to commence extending there must be an increase in its suction force. There is considerable evidence from potato and artichoke tuber tissue and also from *Avena* coleoptile sections that auxins in physiological concentrations bring about a definite increase in the suction force of growing cells. The crucial problem however is the origin of this increased suction force and here various possibilities present themselves. Firstly it could arise from an increased osmotic potential in the cell sap. This possibility has been indicated by observations that applications of synthetic auxins to plants may cause sugars to accumulate and starch to disappear. However such metabolic disturbances only arise at applied concentrations far in excess of the normal natural auxin content and probably have no direct connection with the water intake in extension growth. In any case many direct determinations of the osmotic pressures of extending cells show that there is a marked decrease, such as would be experienced if the total osmotically active material of the cell were diluted by the growth water taken in. This has been shown to happen in the *Avena* coleoptile and in potato tuber tissue. On the other hand in roots the osmotic pressure remains remarkably constant during the extension of the cell. Such observations have suggested the theory that auxin induces active uptake of solutes from the environment and that water uptake and wall extension are a direct result of the augmented osmotic potential. This latter theory lacks direct confirmatory evidence.

The only other way in which osmotic equilibrium can be disturbed is by a reduction in the internal hydrostatic pressure of the cell contents, the so-called turgor pressure of the majority of plant physiologists. A change here can be due only to a change in the properties of the cell wall. Any effect which increases its extensibility will, under a given tension, cause it to stretch. There will arise a consequent reduction in turgor pressure and water will enter the cell. Demonstrations of auxin-induced increases in wall extensibility have been made at intervals ever since the pioneer observations of HEYN, and there are several claims that during the early stages of extension the cell wall actually decreases in thickness, indicating that extension starts by a purely physical stretching of the cell wall. Much controversy has waged over the relative importance of plastic and elastic extensibilities in these effects. Although the data are by no means consistent, growth seems more closely correlated with changes in plastic than in elastic extensibilities. But, as BURSTRÖM has pointed out for roots, the elastic limit is never likely to be exceeded in the stretching of normal cells and so a plastic extension under turgor is unlikely to be a phase of normal growth. It seems likely that misconceptions may have arisen in the past by the glib assumption that the cell wall is homogeneous and extending over its whole length. In actual fact, as FREY-WYSSLING has shown, extension may be confined mainly to the tip of a cell and the « average » properties of the cell walls in any tissue may have little significance in the growth process.

However, during growth under auxin action, there are marked changes in cell wall properties and many theories of auxin action have sprung therefrom. A claim that auxins increase the swelling capacity of mucilages and gums suggested a similar action on the pectic intermicellar colloids of the cell wall but such claims have not been subsequently substantiated. One of the earliest theories proposed that auxin loosens the bonds holding together the cellulose microfibrils in the cell wall lattice, thereby allowing the meshes of the lattice to be opened under the given tensions. Subsequently more microfibrils are inserted in the enlarged interstices but no auxin action was envisaged here since calculations of auxin concentrations in the cell suggested that they were too low for a direct action on cellulose-incorporating enzymes. Recently, however, interest in cell wall synthesis has been revived. The use of carbon isotopes has indicated that auxins have no influence on the rate of incorporation of carbon in the cell wall but, on the other hand, there is evidence that auxins have a direct effect on the metabolism of pectins. Since pectins may be important components in the intermicellary colloids the whole question of a direct action of auxins in cell wall metabolism is reopened.

These considerations do not exhaust the suggestions concerning auxin action in the water relations of the cell. There are observations, some of quite recent date, that auxins can induce the uptake of water by a cell from hypertonic solutions of sugars, eg. mannitol. This effect is explained by a non-osmotic active uptake coupled under the influence of auxin to an energysupplying respiratory process. BURSTRÖM has justifyably criticised this in view of the relative crudity of the associated technique and the phenomenon must therefore be regarded as unproven. However the idea is by no means dead since considerable work shows that the auxin-induced water uptake in tuber tissue is prevented by low concentrations of respiratory poisons such as iodoacetic acid and potassium cyanide, strongly indicating its tight coupling with respiratory energy provision.

Others have seen in changes in the permeability of the cell membranes to water a possibility of direct auxin influence on growth. Considerable data have accumulated on auxin-induced permeability changes in a variety of both growing and mature tissue. Both decreases and increases have been observed, even in the same tissue and with the same technique. Such discrepancies have helped little with the main problem. But even a considerable increase in water permeability induced by auxin cannot be considered to have much connection with the mechanism of auxin action unless it can be shown that the rate of water entry into a cell is limiting the rate of extension growth. Recent calculations by LEVITT prove conclusively that this is by no means so and that the resistance of the cell wall to extension is clearly the limiting factor.

On the whole then the evidence still seems to be in favour of an action of auxin on the cell wall, most likely an indirect biocheminal action requiring the participation of an energy-providing respiration system.

Auxin action in biochemical systems.

Possible auxin action in Thimann's « master system » switched attention specifically from water uptake and cell wall extension to basic metabolic processes in the growing cell. Oxidative respiration immediately suggested itself and it is to James BONNER that we owe the first demonstration of its close coupling with growth. But many investigators favoured the idea that growth and auxin action were closely associated with a particular partial process of respiration. The pioneer work of THIMANN and his collaborators claimed to have identified this with the Kreb's acid cycle since conditions, tending to reduce the activity of this cycle, viz. differential inhibitors such as iodoacetic acid, phenyl mercuric compounds, etc. all greatly inhibited growth to a much greater extent than they inhibited gross respiratory exchanges. These inhibitions were also prevented by substances promoting activity in the cycle, *i. e.* the Kreb's cycle acids. Furthermore under conditions favourable to the Kreb's cycle, β-indolylacetic acid produced a definite though small stimulation of respiratory rate. The development of this theme by various groups of workers has provided us with a mass of complex interactions between specific respiratory inhibitors and auxins in growth and respiration of coleoptiles, epicotyls, roots and tuber storage tissue. Results have not always been consistent and an unbiassed sifting of all the data to date would find in them no conclusive evidence that extension growth is coupled specifically to the Kreb's cycle and even less that auxin exerts its action somewhere in that cycle. With regard to the stimulation of respiration by auxins, many of the effects first obtained could be regarded as *results* of growth, since the material had increased in size by an equivalent amount during the respiration determinations. Such a correlation between respiration and size has been fully established in the extending cells of roots. Nevertheless, carefully planned experiments by ANKER at Utrecht have established a definite stimulatory effect of auxins on the respiration of coleoptile sections and this has been interpreted as a direct action on a respiratory enzyme system.

It has been suggested from time to time that respiration and growth might be stimulated by auxin if it increased the rate of mobilisation of sugars from starch. It has been known for a long time that application of high doses of synthetic auxins may cause this to happen but the associated growth effects are gross distortions or even complete inhibitions and there is consequently grave doubts whether such responses are connected in any way with normal auxin action. Certainly early claims that the activity of amylolytic enzymes could, under certain conditions *in vitro*, be put up by auxins, have never been substantiated. On thermodynamic grounds, however, it has been pointed out that the energy supply of normal respiration is vastly in excess of that required in extension growth and that a small stimulation of that gross respiration by auxins would not on this score be likely to stimulate growth.

This pioneer work on auxin action and the Kreb's cycle started a fashion in the use of differential inhibitors in auxin mechanism studies. Thus arsenate, which interferes with phosphate metabolism in the cell, induces, at suitable concentrations, a complete inhibition of growth of *Avena* coleoptile sections without affecting oxygen uptake and this inhibition is completely removed by added phosphate. It is also claimed that the respiratory stimulation by β-indolylacetic acid is prevented by the same arsenate concentrations. On such evidence a theory has been formulated that growth control by auxins must be via the supply or utilisation of respiratory energy in the form of high energy phosphate compounds. Such sweeping conclusions are not justified on such indirect evidence. Obviously anything which blocks the flow of energy into syntheses will eventually stop growth processes but the evidence will not yet allow us to conclude that auxin controls growth by directly regulating that flow.

A plethora of differential inhibitors have from time to time been similarly used to analyse the interrelations of growth and respiration and to fit auxin into its proper place in this complex jig-saw puzzle. The attack to date has been too empirical and superficial to inspire any confidence in the far-reaching conclusions drawn from the results.

The relationships between molecular structure and auxin activity.

Before the last world war brought research virtually to a standstill in this field the basic structural requirements for auxin activity had been worked out. Briefly they were a ring system with at least one double bond and a side chain terminated by a carboxyl group. Since the war hundreds of new auxin molecules have been synthesized and tested for relative activity and the generalisations that have thereby come into being have given rise to important theories of auxin action. Naturally the data are so extensive that no general survey can be attempted here. We can but indicate only those general aspects of structure that have given rise to definite theories.

The first theory of any value came from observations of VELDSTRA that activity was associated with a proper balance between the affinity of the ring system for lipoids and that of the carboxy groups for water. This suggested action at a lipoid/water interface and led to the theory of growth control by permeability regulation in the lipoid-rich membranes of the cell. This theory fell when no similar correlations could be observed between auxin activity and permeability effects on the membranes of red beet cells. Nevertheless action at an interface was not abandoned, especially since it was shown that maximum activity in the range of molecules studied seemed to be closely associated with an effective orientation of the carboxyl dipole in a position perpendicular to the plane of the ring. The binding of the auxin onto a surface in such a way that the carboxyl group can fit into a receptor, possibly of a specific shape, was therefore held to be essential for its action. The nature of the surface and the specific receptor could not be indicated.

However, more intensive considerations of the effects on activity of various group substitution in side chain and ring have given rise to more detailed speculations as to the nature of the auxin-receptor combination. One has postulated that union must be between three specific spots on the growth centre and three groups of the auxin molecule. These groups, the carboxyl group, the ring system and a hydrogen atom, must therefore have a certain orientation to the three receptive spots, each specific to its own auxin group, before the auxin will fit on and exert its action. Again the nature of the receptor cannot be indicated from the available data. A second theory required combination to take place between receptor and only two points in the auxin molecule, the carboxyl group and one of the positions in the ring system ortho to the side chain or carboxyl group. This theory identifies the receptor with a protein and suggests that the carboxyl combines with an amino group of the protein while the ortho position of the ring is substituted by a cysteinyl group in the protein.

It is, however, fair to point out that the chemists themselves are far from agreeing on the chemical interpretation of the available facts, especially since the relative activity adduced for any molecule often varies with the physiological test applied. This indecision is inevitable so long as adequate physiological work lags far behind the flow of new chemicals.

The interactions of auxins with other chemicals.

Naturally this recent emphasis on molecular structure and activity has reminded plant physiologists of the success acheived in the elucidation of growth processes in micro-organisms by the study of antimetabolites. Much attention has recently been paid therefore to the investigation of possible anti-auxins, *i. e.* chemical compounds, either naturally occurring or synthetic, which may compete with auxins for the growth centres and thereby prevent or seriously hamper its actions. Many such compounds are now known and, as would be expected, most of them resemble the auxins closely in molecular structure, although differing in essentials. The isobutyric homologues of the active acids are good examples. So far, however, a study of these homologous antagonists of auxins have done little more than confirm the molecular requirements already established in direct auxin studies. Similar work on possible synergists, which, while inactive by themselves, may greatly augment auxin action, has so far merely indicated that auxins may combine at centres in the cell where they cannot control growth and that they may be released therefrom by the competitive action of homologous nonauxin synergists.

Some promise of success is given by studies of the interactions in growth between auxins and other natural metabolites. Thus the vitamin ascorbic acid has been claimed to « activate » auxins in *Avena* coleoptile growth. The deduction that this is because auxin must be maintained in a reduced condition for activity seems unlikely from chemical and other considerations. Synergistic effects with nicotinic acid in the growth of etiolated peas still need further study as does the complex interactions of auxins and adenine in the growth development of buds. Certain antimetabolites of nucleic acid synthesis, eg. thiouracil, inhibit seedling growth and, it is claimed, block auxin action. Auxins are thereby visualised as regulating protein synthesis on these nucleoprotein « jigs ». These preliminary observations need further consideration and confirmatory work.

This new approach has brought to the fore the question of naturally-occurring anti-auxins which take part with auxins themselves in the delicate control of growth. Already many natural growth inhibitors are known and the possibility of their functioning as anti-auxins is being actively investigated. Some may even be homologues of β-indolylacetic acid as forcast by Stewart's early isolations of an inhibitor which could be converted to an auxin. This conjures up a stimulating picture of growth control by enzymic auxin-inhibitor interconversions in the plant. These substances will undoubtedly contribute much to our knowledge of auxin action.

Time has not permitted a consideration of the vast mass of data dealing with the toxic action of synthetic auxins applied in high concentrations. There are some physiologists who would hold that such activity has little if anything to do with the action of natural auxins in the control of growth. On the other hand the basic toxic action of these various auxins, once they have entered the cell in sufficient quantities, runs closely parallel to their activity in auxin tests and it is reasonably safe to conclude that these two activities have something in common. No doubt we have much to learn of natural auxin action from a study of these toxicities.

A MECHANISM OF AUXIN ACTION

by Toshio Yamaki
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The physiologists have devoted much attention to the interrelationships of elongation and respiration. But recently, experimental results have accumulated suggesting that an auxin, especially IAA, may not be an accelerator of the respiration system which is responsible for the supply of growth energy. The following investigations afford a new field to the study of the action of IAA. IAA may increase both the elongation of tissues and the intake of CO_2, when CO_2 exists in their environment.

In the electron microscopic observation of the cell wall, etiolated *Avena* coleoptile cylinders prepared from growing zone were treated by KOH and shadowed with Cr. The microfibrilar network of the primary wall, which has elongated 17 hrs. on the surface of 5×10^{-6}mol IAA at 25°C in the dark was compared with that elongated on distilled water and with that of the cylinders which have just been cut off from the coleoptile. But no obvious difference of the microfibrilar structure was found among 70 micrograms obtained. From this experiment it is probable that the intussusception of new microfibrils may occur during the elongation of the cell wall.

5 mm coleoptile cylinders (initial length) when floated on distilled water decrease their dry weights, but their dry weights increase when floated in 5×10^{-6}mol IAA solution. In the average of 10 experiments treated with IAA, an increase of 1.2 mgr of dry weight per 100 cylinders was obtained during 3.77 mm elongation of each cylinder, and in the average of 6 experiments treated with water, 0.6 mgr of decrease was obtained during 0.84 mm elongation of each cylinder. In an exceptional case in the treatment with IAA, 6.4 mgr of increase was mentioned during 4.96 mm elongation. In IAA treated cylinders their dry weights increase during elongation, but the weight of the absorbed IAA is very small compared with the increase of dry weight of the cylinders. This means that some synthetic reactions accompany elongation in the intake or fixation of some substance (s) (perhaps CO_2) from their environment. According to the elementary analysis, the quantity of C of IAA treated cylinders has increased compared to the initial quantity of C, but that of water treated cylinders has decreased.

Auxin free etiolated cylinders of 5 mm long, were placed in recently designed vessels of Warburg's manometer containing borax-potassium phosphate or borax-boric acid-NaCl buffer solution of pH 7.0, with or without the addition of IAA at the concentration of 5×10^{-6}mol. The vessels of these respirometer were designed to make the cylinders respire in aereted conditions and to measure the variations of the gas pressure and the elongation of the cylinders at the same time. The experiments were carried in the light and in the dark at 25°C. Of the 3 vessels used in each experiment, the larger gas absorptions were observed in the vessels, which contained KOH (A) or diethanolamine (B) in a compartment of the vessel, but a small absorption was yet observed in the vessel which contained neither KOH nor diethanolamine but water (C) in its compartment. And the elongations of cylinders in the vessels B and C were much larger than that of the vessels A. This negative pressure of the vessels C was not due to an excessive absorption of oxygen but perhaps to the consumption of CO_2 by the coleoptile cylinders themselves. As a proof, the negative pressure was still observed when sucrose was added as a substrate to the buffer solution of the vessels C. IAA increases the gas absorption of these vessels and this increase was about 15-20 % in the vessels C, but the increase of the vessels A was nearly the same as the increase the vessels C. And the accelerated elongation of the cylinders in the vessels C was much larger than that of the vessels A. These experiments indicate (1) that the larger elongation takes place rather in the vessels, from which no CO_2 is removed, than in the vessels, from which CO_2 is removed by KOH, (2) that isolated cylinders can absorb CO_2 from their environment not only in the light but in the dark, and (3) that IAA acceralates this CO_2 intake and the elongation of the auxin free cylinders at the same time.

Auxin free cylinders of 5 mm long were floated on borax-potassium phosphate buffer solution of pH 7.0 and were elongated under the blow of CO_2 free air or CO_2 containing air, 3 hrs, at 25°C in the dark. They elongated a little in normal air and in the air which contained 7-10 % CO_2, but hardly elongated in CO_2 free air. On the same buffer solution but containing 5×10^{-6}mol IAA, the accelerated elongations of these cylinders were much larger in the two former CO_2 conditions than in the later CO_2 condition.

ON THE MECHANISM OF THE ACTION OF AUXINS

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Besides a variety of externally visible, generally known effects of the application of growth substances (cell elongation, root initiation, early cambial activity, delayed leaf abscission etc.) also a number of internal reactions of the most different character can be induced by these substances (increase of the plasticity and of the elasticity of the growing cell wall, change of the permeability of the protoplasm, increase of the endogenous respiration etc.). In all these cases the kind of the effect and even its sign is largely dependent on the concentration of the growth substance applied (so called concentration effect).

None of these effects, however, readily can be considered the « primary reaction » proper on growth substances.

Further a great variety of chemical compounds shows growth substance activity. All these « auxins » (1) have particular properties in common, but they all lack redox qualities and none of them seems apt to act as prosthetic group of some desmolytic enzyme. Moreover, the concentration effect, yielding an optimum curve for the ratio activity/ log. concentration, is incompatible with such a concept, that only allows a saturation curve (cf. the resynthesis of the yellow Warburg' respiratory ferment from lactoflavin and the apo-enzyme). All auxins, however, are compounds with more or less specific polar qualities, which suggest an activity in lipidic boundary layers.

On the other hand sligth changes in the molecular structure make many auxins turn into hemi-auxins or even into anti-auxins, many of the latter possessing an unsaturated lactone-ring that highly enhances the chemical reactivity of the substance.

It is suggested and moved for discussion that auxins act as sensitizers on amicroscopic lipidic interfaces of the protoplasm. The spots, where the numerous subsequent one-step reactions of the metabolism take place, must be separated in space and the metabolites must follow a certain direction given in the protoplasmatic structure, otherwise the metabolic mechanism would run chaotically. This spacing over amicroscopic distances along the surface of protein molecules requires molecular sheets, insoluble in water, that separate and screen off the subsequent reactive spots from each other, allowing only the adequate substrates to be adsorbed to their specific spots. It is supposed that these screens consist of — perhaps monomolecular — films of protein-bound lipids (phosphatides).

According to this hypothesis auxins would act as openers or shutters (dependent on their chemical nature and concentration) of these lipidic « curtains ». Hemi-auxins possibly would only have this property either to a much less extent, either for a smaller number of particular screens. Anti-auxins, however, perhaps can do both : operate the screens and block reactive spots of the enzymes concerned.

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(1) I would prefer the name « auxinoids » for these substances, in order to preserve the term auxin for Kögl's auxentriolic acid (auxin-a) and its lactone and for auxenolonic acid (auxin-b). If auxin and auxinoids had not been discovered until at present, I wonder whether they still would be baptized « growth substances », viz. « auxins ».

UEBER DEN WUCHSSTOFF DER HAFERKOLEOPTILE

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Die chemische Natur des Wuchsstoffes der Pflanzen ist noch nicht restlos geklärt, nicht einmal für das am haufigsten untersuchte Pflanzenorgan, die Haferkoleoptile, für das teils Auxin a, teils Indolessigsäure als Wuchsstoff angegeben wird. Es zeigte sich, dass der durch Diffusion aus Koleoptilspitzen erhaltene Wuchsstoff gegen Behandlung mit heisser HCl empfindlich, gegen eine solche mit heisser NaOH dagegen beständing ist. Dieses Verhalten schliesst Auxin a als den natürlichen Wuchsstoff der Koleoptile aus ; es stimmt aber überein mit dem der Indolessigsäure. Weitere Versuche zeigten jedoch, dass der natürliche Wuchsstoff auch mit dieser nicht identisch ist oder doch nur zu einem Teil daraus bestehen kann. Im Chromatogramm traten nämlich zwei Wuchsstoffe auf, einer an der Stelle der Indolessigsäure und ein weiterer mit einem kleineren Rf. Dieser letzte Wuchsstoff macht vielleicht die Haupmasse des natürlichen Wuchsstoffes der Koleoptile aus ; gegen die mit dem Chromatographieren verbundenen Manipulationen scheint er sehr empfindlich zu sein. Chemisch steht er wohl der Indolessigsäure nahe. Dieses Ergebnis von der Anwesenheit zweier Wuchsstoffe stimmt überein mit den Befunden von Bennet-Clark und Kefford (1953. *Nature,* **171**, 645) und Lexander (1953. *Physiologia plantarum,* **6**, 406) an anderen Pflanzenarten.

Résumé. — Au cours d'essais d'identification des substances de croissance existant dans le coléoptile d'Avoine, au moyen de la chromatographie, il a été possible de mettre en évidence, d'une part l'acide indolacétique, d'autre part une seconde substance, chimiquement analogue bien que différente, et qui constitue vraisemblablement la majeure partie de l'auxine naturelle présente dans cet organe.

EXPERIMENTAL EVIDENCE OF A COMPETITIVE ACTION OF ASCORBIC ACID IN REGARD TO INDOLACETIC ACID

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Previous researches of the same Autors have shown that AA, when used at concentrations usually found in green leaves exposed to light, inhibits the action of IAA. The results of the experiments here related lead the Autors to the idea of a probably competitive nature of this phenomenon.

The Autors have found that when excised, not decapitated *Avena* coleoptiles are put on agar, the amount of IAA diffusing from the coleoptiles into agar blocks in distinctly higher for those coleoptiles whose tops have been treated with a lanoline paste containing AA. This observation finds a close correspondence in the fact that excised coleoptiles on agar plates show increased growth when their tops are treated with lanoline-AA at concentration 1000 ppm. On the other hand, none of the two experiments is successful when decapitated coleoptiles are used. These results seem therefore to suggest that, following the treatment with AA of the hormone producing center, a larger amount of IAA is set free and can diffuse from the tops downwards to the elongation zone, where a stimulation of growth is thus determined. The same results may explain, at least at same extent, the data of others Autors who noticed a stimulating effect of AA on growth. It has however to be pointed out that even the Autors of the present work have observed a slight stimulating action of AA, but only at very weak concentrations, noticeably lower than those found in green leaves ; a fact which indicates that the antagonism displayed by AA in regard to IAA is of the same kind of that typical of weak auxins.

Similar results were obtained by the Autors through free auxin determinations in lupine leaves infiltrated with AA, or with tryptophane first, and successively with AA ; in both cases the amount of extractable free auxin was higher for the AA treated leaves than for the controls (infiltrated, respectively, with buffer solution or with tryptophane alone).

A third series of experiments, comparable to those showing the increase in growth in the *Avena* coleoptiles with tops treated with AA, has been carried out on castor bean cuttings. In this material a better rooting was observed when the upper portion of the cuttings had been treated with lanoline-AA at low concentrations, the result being thus similar to the one induced by the treatment of the lower portion with an IAA solution.

THE ACTION OF 2,4-D ON DEAMINATING ENZYMES

by Fr. Moewus

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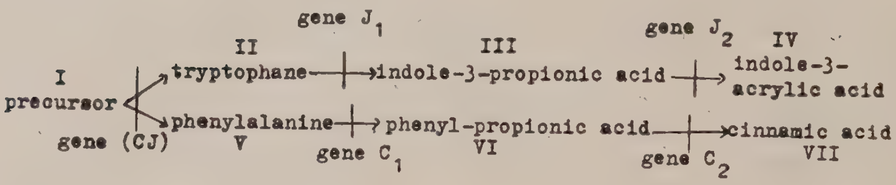
Zygote germination of the unicellular green alga *Chlamydomonas eugametos* consists in meiosis and formation of four genes, a process dependent on the formation of two specific hormones. By means of biogenetical methods the biosynthesis of these hormones could be traced as follows :



In a standard test, the germination rate was determined and found to give a 50 % value after 21 hrs. The addition of IV (10^{-11} g/m1) or VII (10^{-11} g/m1) results in a stimulation from 21 to 17 1/2 hrs. IV and VII, fed simultaneously, give a still higher stimulation effect (14 hrs.), an effect understandable if IV and VII are supposed to regulate two independent but succeeding processes during germination. 2,4-dichlorphenoxyacetic acid (VIII, 10^{-8} g/m1) acts in the same way as IV or VII. However, there is no stimulation at all if it is given simultaneously with IV or VII. Moreover, a mixture or IV, VII, and VIII results in a retarded germination (27 hrs.). These facts suggest that 2,4-D has an indirect influence on the action of production of the two germination hormones.

In order to support this suggestion enzymatic experiments were carried out. $\frac{(CI)^{\circ}}{(CI)^{\circ}}$ -zygotes are able to transform : II to IV, III to IV, V to VII, VI to VII, but not I to II and I to V. Each transformation was investigated in the presence and absence of 2,4-D (10^{-8} g/m1). Zygote brei was incubated for 24 hrs., in the dark, at 25 °C., at pH 7.0, and the output of the enzymatically transformed II, V, III, VI was measured quantitatively by a biological assay. VIII did not have any influence on the enzymatic reductions of III to IV, and VI to VII. However, the deaminations (II to III, and IV to V) were stimulated in the presence of VIII. It could not be finally decided whether the deamination goes via an intermediate step (the corresponding pyruvic acids).

Doubtless there are deaminating processes involved in the formation of the growth hormones in higher plants. Generalizing the results found with *Chlamydomonas* zygotes we might suggest that the application of 2,4-D results in a stimulation effect. Depending on the level of growth hormones present, usually the application of 2,4-D results in an over-production of growth hormones which can be noticed as the well-known inhibition effect.

THE EFFECT OF MALEIC HYDRAZIDE ON INDOLEACETIC ACID OXIDASE ACTIVITY AND GROWTH

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Maleic hydrazide (MH) at 10^{-5} to 10^{-2}M stimulates the rate of enzymatic oxidation of indoleacetic (IAA) by IAA oxidase isolated from etiolated pea stem tissue ; the rate is doubled at 1.25 × 10^{-3}M. MH is equally effective as the free acid or as the potassium or diethanolamine salt. The optimum acidity for acceleration lies at pH 5.6 and the optimum temperature 34°C. The MH effect on IAA oxidase can be demonstrated equally well by three independent methods : (1) colorimetric analysis of the IAA concentration with the $FeCl_3$-H_2SO_4 Salkowski of spot size using isopropyl alcohol : NH_3 : H_2O solvent mixture for partitioning and $NaNO_2$-HCL as the colour developing agent ; (3) rate of oxygen uptake in a Warburg respirometer.

The activity of an IAA oxidase preparation can also be increased by floating the intact pea stem tissues on 10^{-3}M MH for 10 hours prior to extraction. The degree of inhibition of pea stem elongation or slit pea stem curvature by various concentrations of MH reflected by an accelerated loss of IAA from the test solution. The mechanism by which MH stimulates IAA oxidase and the possible effect of IAA oxidase activity on growth are discussed.

THE ENZYMATIC OXIDATION OF INDOLE-ACETIC ACID

by Bruce B. Stowe, Peter M. Ray and Kenneth V. Thimann

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The mode of breakdown of indole-acetic acid is in many respects almost as important for plant physiology as the mode of its formation. The « indole-acetic acid oxidase » of higher plants has been intensively studied, but the nature of the breakdown product is unknown. In order to circumvent the difficulties involved in producing an acceptably pure enzyme from higher plants, we have studied an exceptionally active indole-acetic acid oxidizing enzyme which is secreted by the Basidiomycete *Omphalia flavida*. Purification of this enzyme merely requires dialysis of the liquid culture medium, yielding a preparation which can oxidize IAA at a rate of 574 µl. O_2/hr/mg. N_2. The enzyme has not been found to act on any other indole derivative at an appreciable rate. The stoichiometry is also favorable, the data indicating almost exactly one mole of CO_2 produced for each mole of O_2 taken up and each mole of IAA destroyed. These properties make the enzyme preparation especially suitable for an attempt to isolate and characterize the oxidation product. By means of paper chromatography several possible compounds have been eliminated, including indole aldehyde, which is produced upon photooxidation of IAA, and the hydroxylated indoles which have lately been demonstrated in animal materials. The products which would be derived from an oxidation analogous to that carried out upon tryptophan in mammalian tissues, namely δ-aminoacetophenone and its formyl derivative, are also incompatible with the data. The product is a neutral compound, which has been prepared in sufficient quantity for examination. Its infra-red and ultra-violet spectra and certain other properties have been studied and the conclusions derived will be discussed.

AUXIN AND PROTOPLASMIC STREAMING : AN OXYGEN EFFECT

by J. S. Turner, J. MacRae, P. Grant Lipp
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In 1937 Thimann and Sweeney reported a well marked effect of auxin solutions on streaming of protoplasms in the *Avena* coleoptile. Further work and some controversy resulting from this discovery was reviewed by Audus in 1949 (*Biol. Rev.*, 24). We have largely confirmed the work of Thimann and Sweeney, using the staminal hairs of *Tradescantia virginiana*. We have also obtained confirmation of their finding, that the effect of auxin is dependent upon the oxygen concentration of the medium. In high oxygen concentration the activity of the auxin in relation to streaming is largely diminished. Thimann *et al.* explained this as due to a competition for oxygen by respiratory systems, some of which are associated with streaming. We have found, however, that this oxygen effect may be produced by oxygenating auxin solutions in the complete absence of the living cell. The work to date suggests that a photochemical process which occurs in high oxygen concentration changes the auxin molecule in such a way as to diminish its effect on streaming while leaving it unchanged as a growth hormone.

Staminal hairs of *Tradescantia* were mounted in a hanging drop of water or solution and the rate of streaming was measured by a device adapted from that described by Thimann. The hanging drop was equilibrated with air, as it was found that the rate of streaming was independent of oxygen concentration between 1.5 and 100 %.

Solutions of indoleacetic acid at concentrations ranging from 0.1 mgm. to 10 mgm./L. cause a transient stimulation of the rate of streaming. In the continued presence of these solutions the rate returns to the normal within one hour. Concentrations between 10 and 50 mgm/L. have little effect upon the rate and concentrations greater than 50 mgm/L and up to 100 mgm/L cause a transient inhibition of streaming, which again is lost within one hour, when the rate again returns to the normal. These results show a striking parallel with those obtained for the *Avena* coleoptile, the major difference being in the concentration of auxin necessary to bring about similar effects in the two tissues. If we plot the *total effect* of the auxin solution on the streaming, we find that the maximum effect is given for *Tradescantia* by concentrations of near 1 mgm/L, whereas for *Avena* the maximal effect is given by 0.01 mgm/L. The results for *Tradescantia* are closely similar to those obtained by THIMANN and SWEENEY for the elongation of *Avena* coleoptile plotted against the logarithm of the auxin concentration. The effects of auxin upon protoplasmic streaming take place within the physiological range for auxin. Similar results were obtained with indole butyric acid and naphthalene acetic acid. In all three cases the acceleration or retardation of streaming is stabilised by the addition of malic acid or fructose with the auxin. The acid and the sugar added alone have no effect whatever on streaming.

Experiments with phosphate buffers and with organic acids show that streaming is not affected by change of pH from 7-4. The effects described above appear to be specific auxin effects and are not due to the slight change of pH which is brought about by dilution. Nevertheless, the effect of a given auxin solution on streaming is closely dependent upon the pH of the solution. For instance, auxin at 100 mgm/L, pH 4 gives a total effect of —4.9, while the same concentration applied at pH 7 gives a total effect of +5.8.

THIMANN *et al.* have attempted to explain the effects of auxin upon streaming in terms of its effects upon respiratory systems. Like them, we have found that certain respiratory poisons also inhibit protoplasmic streaming in *Tradescantia.* A new finding is that malonic acid has almost precisely the same effect upon protoplasmic streaming when applied over a range of concentrations and pH as it has upon the respiration of slices of carrot tissue. On the other hand, in conformity with some work by THIMANN, we have made the rather curious observation that iodoacetic acid has no effect upon the basal rate of streaming, but inhibits the stimulated streaming brought about by auxin solution. Although auxin may effect streaming through its reaction on respiration, we cannot agree with THIMANN and SWEENEY that the oxygen effect noted by them and confirmed by us is due to a competition for oxygen by respiratory systems. For *Tradescantia* the effect of high oxygen concentration is to move the whole curve of total effect/log. auxin concentration to the right. That auxin concentration which, when applied in air, causes a depression of streaming, brings about stimulation after oxygenation of the solution. Maximum stimulation is given for oxygenated solutions, not by concentrations of 1 mgm/L, but by concentrations of 10-20 mgm/L. For *Tradescantia,* we obtained oxygen effects of this kind over the whole range of the effective auxin concentration, whereas for *Avena,* high oxygen only modifies the effect for solutions causing inhibition. However, for both tissues it is apparent that there are similar oxygen effects, so long as the auxin concentration is greater than 0.1 mgm/L.

We find that the oxygen effect is not dependent on the presence of a high oxygen concentration in the hanging drop containing the living cell, as is clearly required by the hypothesis of SWEENEY and THIMANN. An auxin solution whose activity has been markedly reduced by oxygenation has the same effect on streaming when used directly as it has after equilibration with air. To obtain a marked oxygen effect it is necessary to pass the gas for only a few minutes. For instance, in one experiment oxygen was passed through a pure solution of auxin at 500 mgm/L for periods of 3, 6, 12, 24, 48, 92 minutes. After each period a part of the solution was equilibrated with air and used for the test. The untreated solution of this very high concentration brought about a rapid, complete and irreversible inhibition of streaming. Most of the activity was lost after a few minutes oxygenation and the solution which was treated for 92 minutes with oxygen behaved as though its auxin concentration was less than 0.5 mgm/L. These treated solutions showed no recovery as regards their effect on streaming if they were preserved over air for a further 96 hours.

The effect of oxygen on the auxin solution does not take place in the dark. It requires diffuse daylight and will also take place with artificial light in the range 365-436 mμ. Simple aeration of the solution in daylight or in U. V. 365 mμ brings about no change in the properties of the auxin solution.

Naphthalene acetic acid has much the same effect upon streaming as has indoleacetic acid, but it shows no oxygen effect whatever. The oxygen effect is only shown, in our experience, by indole-acetic acid and indolebutyric acid. It is not, however, a direct photoxidation of indoleacetic acid to indole-aldehyde, as described by DENFER *et al.*, as oxygenation of auxin solutions bring about no change in their growth promoting activity (*Avena* test). Our evidence suggests that the oxygen effect is not due to an impurity in the oxygen stream, nor to the destruction of an impurity present in the indoleacetic acid itself. The form of the curve relating auxin concentration to the rate of streaming and the effect of pH upon this curve suggest that the effects on streaming are due to the auxin itself and not to traces of an impurity. This is supported by the results obtained with naphthalene acetic acid.

Treatment of an auxin solution with dilute hydrogen peroxide also reduces its activity in relation to streaming. However, using the very sensitive luminal test we can detect no trace of hydrogen peroxide when a pure auxin solution is oxygenated. We have to admit that up to date chemical and physical tests of such oxygenated auxin solutions have failed to reveal any changes in the molecule. These tests include :

1. pH and titration curve.
2. Ultra-violet absorption spectrum.
3. Surface tension.
4. The Tang-Bonner spot test.
5. The Hopkins Cole test.
6. Free hydrogen by the nitrosyl test.
7. Paper chromatography.

If the auxin solution is first mixed with malic acid and then oxygenated, there is no change in its properties ; it continues to give the normal effect, stabilised by the presence of the acid. One line of attack which is being pursued at the moment is to see whether the malic acid is itself changed while protecting the auxin from the effect of the oxygen.

THE INITIAL EFFECT OF HETEROAUXIN ON THE WATER UPTAKE BY POTATO TISSUE

by L. Brauner

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Reinders' observation that the aerobic water uptake of plant parenchymes is conspicuously enhanced by small amounts of IAA has been confirmed by several workers. However, considerable disagreement still exists as to the interpretation of the factors involved in the reaction. Reinders herself mainly considers the possibility of a new-formation of osmotic substance and, as an additional factor, a softening of the cell walls. Commoner, on the other hand, suggests the participation of anomalous osmosis, linked to the respiratory ion uptake. This view was rejected, however, by Overbeek, because auxin stimulates the water absorption also in dist. H_2O. Since no evidence for auxin-induced anatonosis could be found either, Overbeek attributes the effect to an anomalous osmotic mechanism, acting directly upon the water molecules, and possibly aided by a simultaneous reduction of the wall tension. The absence of anatonosis was also confirmed by Levitt. As thermodynamic considerations led Levitt to question the participation of anomalous osmosis as well, he concludes that the auxin-induced water uptake has to be attributed exclusively to an enhancement of the cell wall tensility.

This was the general situation when we began to re-examine the problem in 1949. Since in none of the previous work the elastic conditions in the tissue were actually examined, it was particularly important to measure quantitatively the effect of IAA upon the tensility of the cell walls in the water absorbing parenchyme. For this purpose prismatic rods of tissue first were exposed to the experimental solutions for definite periods of time. Then they were plasmolyzed with glycerol and mounted to a stretching apparatus permitting the application of definite stresses. By measuring the reversible and the permanent extension of the specimens comparative values were obtained for the total (T) and the plastic tensility (P) of the tissue. It was found that in the presence of oxygen 10 p.p.m. IAA significantly increased both T, and more particularly, P. This change becomes noticeable already after 3 hr. and it reaches its maximum at the end of the first day. At that time T and P of the auxin samples exceed the corresponding values of the water controls by 9.7 % and 19.2 % respectively. Under anaerobic conditions this effect fails completely to develop. It therefore is believed that IAA affects the mechanical properties of the cell wall *indirectly* by modifying the respiratory metabolism. — One may question whether the observed changes in tensility are not the consequence rather than the cause of the concomitant increase of the cell volume. However, this alternative is ruled out by earlier observations that in auxin-free media the increase of strain due to the rising turgor, far from plasticizing the extended cell wall, tends to harden it. Thus, a direct enhancement of the suction potential (S.P.) of the tissue by an auxin-induced lessening of the wall pressure (W) appeared quite conceivable, even if the osmotic value of the cell content remains unchanged. In order to test this possibility gravimetric determinations of S.P. were carried out after definite periods of water absorption in dist. water and in 10 p.p.m. IAA respectively. It appeared that from the 12th hour on the S.P.-values of the auxin samples (a) increasingly exceeded those of the water controls (c). This effect, too, developed only in the presence of oxygen. After 24 hr. S.P. (c) was found to be 0.40 atm., S.P. (a), 0.67 atm. From cryoscopic measurements the corresponding values of the osmotic concentrations (O) were obtained : O(c) = 5.19 atm. and O(a) = 5.09 atm. By inserting these figures into the conventional suction potential equation S.P. = O — W one finds : W (c) = 4.79 atm. and W(a) = 4.42 atm. From this calculation the auxin-induced reduction of W appears to be 7.7 %, while the direct determination of the respective tensilities showed T(a) to exceed T(c) by 9.7 %. Since two very different methods, thus, yield values of the same magnitude, it seems admissible to ascribe the main part in the enhancement of S. P. to the softening of the cell walls.

There is, however, yet another factor involved in the reaction : a considerable reduction of the diffusion resistance which, particularly during the first stages of the absorption, noticeably increases the velocity of the water intake. This change shows itself in the much steeper trend of the determinant curves of the S.P.-measurements, indicating that the auxinated tissue absorbs more water from the lower, and loses more water to the higher gradations of the concentration series than the controls do under identical diffusion pressure. This effect which also develops only under aerobic conditions, dominates the reaction during its first hours, before the softening of the walls has become effective. During this phase the excess of water uptake produces in the auxin samples a transitory depression of their S.P. below the level of the water controls.

Cryoscopic measurements of the cell sap concentration confirmed the findings of previous workers that the absolute amount of osmotic substance does not increase during the auxin-induced water uptake.

In conclusion it has to be stressed that both tensility- and permeability reaction were found to become significant already within a few hours of IAA-application. This observation is in contrast with Reinders' and Hackett et Thimann's assertion that a time lag of 2 days precedes the setting-in of the auxin-induced water uptake. The discrepancy is probably explained by the fact that these authors intentionally exclude the « initial » phase of the process from their observations by pre-saturating their material before experiment.

THE EFFECT OF INDOLEACETIC ACID AND OF ASCORBIC ACID ON WATER UPTAKE AND ON WATER HOLDING CAPACITY

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Ascorbic acid (AA) and indoleacetic acid (IAA) at physiological concentrations display, as shown by the previous work of the same Authors, an opposite action on the plant cell. The effect of the two substances depends from their concentration : so IAA at high concentrations may inhibit growth, or, on the other hand, low concentrations of AA may stimulate the same process. When both substances are present, the final result depends from their individual concentration as well as from the ratio of that of the one of them to that of the other. In a general way, it may be said that, at the concentrations usually found in tissues, IAA is endowed of a stimulating, and AA of an inhibiting activity.

The Authors complete and extend the data obtained by themselves and by others showing that those concentrations of IAA which stimulate water uptake, as it appears from a series of experiments on avena coleoptiles, on castor bean hypocotiles, on pea internode sections and on lupine and bean leaf disks. Other experiments, on entire avena seedlings and on castor bean plants, in which the processes of *guttation* and of bleeding wass studied, lead to the same conclusion : in both cases increased water uptake by the roots was shown by the increased activity of *guttation* or bleeding. On the other hand, Ascorbic acid induced, in all these materials, an effect opposite to that of IAA, and it inhibited water uptake in avena coleoptiles, castor bean hypocotiles, pea internode sections, and leaf disks from lupine and peas, as well as guttation in avena seedlings and bleeding in castor bean plants. The process of water loss in tissues previously treated with IAA and AA, and dryied at temperatures varying between 20 and 100 centigrades has been the object of a further series of experiments, in which avena coleoptiles, lupine and castor bean hypocotiles and lupine leaves were used as materials.

Water loss was slacked down by the treatment with IAA, while it was accelerated by the treatment with AA, for concentrations of both substances similar to those active on growth and water uptake. An analogous difference in dehydration speed was found to exist between the green and the albine leaves of *Acer Negundo,* naturally containing a higher or, respectively, lower content in AA.

These data are interpreted in agreement with the views previously expressed by the same Authors about the action of growth regulators on cell plasm.

DER EINFLUSS VON WIRKSTOFFEN AUF DIE PLASMAPERMEABILITAT

von Hermann GUTTENBERG

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In einer Studie über die Mechanik der Schlafbewegung konnten wir feststellen (GUTTENBERG und KRÖPELIN, 1947), dass Auxin, das einseitig auf das Laminargelenk von *Phaseolus coccineus* aufgetragen wird, eine negative Krümmung hervorruft. Da diese auf einer einseitigen Schwellung der Zellen des Gelenkpolsters beruht, musste man annehmen, dass Auxin eine Erhöhung der Wasserpermeabilität bewirkt. Um die Richtigkeit dieser Annahme zu prüfen, untersuchten wir den Einfluss des Auxins auf die Wasserpermeabilität der violetten Epidermiszellen der Blattunterseite von *Rhoeo discolor* (GUTTENBERG und BEYTHIEN, 1951). Wir arbeiteten nach der Plasmolyse-Deplasmolysemethode. Als Plasmolytikum diente eine 0,3 molare Lösung von Mannit, als Deplasmolytikum eine 0,2 molare. Die geprüften Wirkstoffe waren : B-Indolylessigsäure (IES), extrahiertes Avena-auxin Phenylessigsäure, Cumarin, Parasorbinsäure u.a. in verschiedenen Konzentrationem.

Bei Verwendung von IES ergab sich, dass die Konzentration 10^{-5}-10^{-7} g/ccm die Deplasmolysezeit in steigendem Masse gegenüber einer Wasserkontrolle verkürzt. Während in Wasser die vollständige Deplasmolyse erst nach 35-50 Minuten erreicht war, erfolgte sie in IES 10^{-7} g/ccm schon nach 20-27 Minuten. Die Konzentration 10^{-4} ruft Dauerplasmolyse hervor, bei Verwendung von 10^{-3} wird das Plasma zerstört. Andere Auxine hatten im Prinzip die gleiche Wirkung, Blastokoline dagegen wirkten entgegengesetzt. So verzögert z. B. Cumarin 10^{-4} g/ccm die Deplasmolyse um etwa 15 Minuten. KCl fördert den Wassereintritt zwischen 10^{-3} -10^{-6} g/ccm, $CaCl_2$ hemmt ihn bei 10^{-2} -10^{-3} g/ccm. Kombiniert man KCl 10^{-6} mit IES 10^{-4}, so tritt nicht mehr Dauerplasmolyse ein, vielmehr überwindet die fördernde Wirkung des K die in dieser Konzentration hemmende der IES. Cumarin 10^{-4} und IES 10^{-7} gleichen sich in ihrer Wirkung fast aus, ebenso KCl 10^{-6} und $CaCl_2$ 10^{-3}.

In einer weiteren Untersuchung (Guttenberg und Meinl, 1952) prüften wir, ob die Wirkung der Wuchsstoffsäuren eine spezifische ist, oder ob dabei die mit der Konzentration wechselnden P_H Werte von Bedeutung sind. Es ergab sich bei Zusatz von Puffergemischen, dass der Einfluss der IES auf die Wasserpermeabilität tatsächlich spezifisch und nicht durch das P_H bedingt ist. Verwendet man Säuren oder Puffer allein, so ergibt sich ein Maximum der Wasserpermeabilität bei P_H 5,4-5,8, während maximal wirkende IES (10^{-7} g/ccm) ein P_H von 6,2 besitzt. Der P_H Wert der zerstörenden IES 10^{-3} beträgt 3,7, während Säure von gleichem P_H Wert nur etwas verzögernd auf die Deplasmolyse einwirkt.

Die Versuche liessen vermuten, dass die Erhöhung und Herabsetzung der Wasserpermeabilität durch Wirkstoffe darauf beruhe, dass diese den Quellungszustand des Plasmas verändern. Daher wurden in einer weiteren Arbeit (Meinl und Guttenberg, 1952) Modellversuche durchgeführt, die den Einfluss von Wirkstoffen auf Gelatine und Agar prüften. IES hatte auf Gelatine eine Wirkung, die durchaus der glich, die Auxine auf das Plasma ausüben. IES 10^{-7} g/ccm ergab nach 24 Stunden eine maximale Quellung der Gelatine, während die Konzentration 10^{-4} ein Quellungsminimum zeigte und die Konzentration 10^{-3} die Gelatine zum Zerfliessen brachte. Diese Vorgänge fanden auch dann statt, wenn die Lösung gepuffert, war, es lag also kein P_H -Effekt, sondern eine spezifische Einwirkung vor. Säurezusatz allein bewirkt ein Quellungsminimum im isoelektrischen Punkt der Gelatine (P_H 4, 7) und einen Quellungsanstieg nach beiden Seiten dieses Punktes. Gelatinelösungen besitzen ein Viskositätsminimum bei Zusatz von IES 10^{-4}, also bei dem gleichen P_H Wert wie das Quellungsminimum ; bei Säuren tritt es im isoelektrischen Punkt auf. Die Wasserpermeabilität der Gelatine bei Zusatz von IES verhält sich wie die des Protopalsmas : bei der Konzentration 10^{-4} ist sie am geringsten, bei 10^{-7} maximal. Daraus ziehen wir den Schluss, dass die Erhöhung der Wasserpermeabilität des Plasmas durch IES niederer Konzentration auf einer Erhöhung der Quellung (oder

Viskosität) beruht, umgekehrt die Dauerplasmolyse bei der Konzentration 10^{-4} auf Entquellung. Somit verändert IES den Filterwiderstand des Plasmas.

Säuren wirken auf Gelatine umgekehrt wie Auxine. Im isoelektrischen Punkt der Gelatine liegt das Minimum der Wasserpermeabilität, in dem des Plasmas das Maximum. Da im isoelektrischen Punkt Entquellung eintritt, kann der rasche Eintritt des Wassers bei Säurezusatz nicht durch einfache Herabsetzung des Filterwiderstandes bedingt sein, vielmehr müssen besondere Kräfte zwischen Plasmagerüst und den Wasserdipolen wirksam sein. Cumarin wirkt auf Gelatine ebenso entquellend wie auf das Plasma, die Herabsetzung der Wasserpermeabilität wird hier also offenbar durch Entquellung bewirkt. Der Eintritt von Traubenzucker in die Zelle wird bei Gegenwart von IES 10^{-4}-10^{-7} deutlich beschleunigt. Auf Agar übt Auxin keine spezifische Wirkung aus, daher wohl auch nicht auf Zellulosemembranen.

Nach diesen Versuchen betrachten wir die Erhöhung der Wasserpermeabilität des Plasmas durch Auxine als die primäre Wuchsstoffwirkung. Der erleichterte Eintritt von Wasser ermöglicht die rasche Wasserfüllung der sich streckenden Zellen. Die Erhöhung der Dehnbarkeit der Zellmembranen ist ein sekundärer Effekt, eine direkte Beeinflussung durch Auxine liegt nicht vor. Es ist zu vermuten, dass die erhöhte Durchlässigkeit für Traubenzucker bei Vorhandensein von Auxinen den Einbau neuer Zellulosemoleküle in die Zellwand erleichtert.

L'INFLUENCE DE SUBSTANCES ACTIVES SUR LA PERMEABILITE CYTOPLASMIQUE

Des recherches antérieures de Guttenberg et Kröpelin (1947) et de Guttenberg et Beythien (1951) avaient montré que l'auxine et diverses autres substances (acide phénylacétique, coumarine, acide parasorbique, etc.) agissaient sur la perméabilité cytoplasmique. Cette propriété a été mise en évidence par une méthode basée sur la mesure de la durée de la déplasmolyse. L'acide indoleacétique à des concentrations variant de 10^{-7} à 10^{-5} accélère la déplasmolyse. Il en est de même de l'auxine naturelle extraite de l'avoine. Au contraire, la blastocholine et la coumarine ont une action retardatrice. L'action de sels : KCl, $CaCl_2$ a également été étudiée. D'autres expériences de Guttenberg et Meinl (1952) ont montré, grâce à l'emploi de solutions tamponnées, que l'action observée est spécifique et n'est pas due au pH. Les mêmes auteurs ont alors étudié l'action des mêmes substances sur le gonflement de la gélatine et la gélose. Ils ont constaté le parallélisme entre cette action et celle observée dans le cas de la cellule vivante. Suivant les substances essayées, au point isoélectrique correspond soit un maximum, soit un minimum de perméabilité.

En conclusion, l'accroissement de la perméabilité cytoplasmique est le premier effet des substances de croissance alors que l'augmentation de la plasticité de la membrane est un effet secondaire, où l'auxine n'a pas d'action directe. De plus, la perméabilité plus grande au glucose provoquée par la présence d'auxine, faciliterait l'édification de nouvelles molécules de cellulose dans la paroi cellulaire.

GROWTH REGULATORS AND CELL-WALL PROPERTIES OF ROOTS

by Hans Burström

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Growth and water absorption of roots have been inhibited by the addition of mannitol to the nutrient solution. Transferred to a normal nutrient solution such roots show a high degree of plasticity of the cell walls, which is responsible for about one half of the maximum elongation of the cells. The rest involves an active building up of cell walls of a high and fairly constant elastic tension.

If elongation is decreased by the addition of 1-naphthylacetic acid the cell walls maintain their elasticity. If the water absorption of such auxin-treated roots is inhibited no plasticity of the wall is revealed. Withdrawal of calcium from the solution also decreases elongation but gives cells with wholly inelastic walls ; a further decrease by mannitol reveals that the walls are plastic and somewhat elastic. Addition of coumarin causes decreased elongation connected with a total loss of wall elasticity.

Theory. — Cell elongation starts with a dissolution of the wall, which partly appears as a plasticity. The strict concepts of plasticity and elasticity can, however, probably not be applied to walls of colloidal-micellary structure. Excess of auxin increases this dissolution by inhibiting the fixation of stretched walls, which appears as plasticity, turning it entirely into a reversible elasticity. Calcium is responsible for the building up of the elastic component of the wall. — The auxin and calcium-regulated systems may represent different components of the wall or successive developmental stages of one wall structure. — Coumarin seems to interfere in the calcium-regulated system and not with auxin.

LES MOUVEMENTS DE CROISSANCE DES TIGES VOLUBILES ET LA PESANTEUR

par Lucien **Baillaud**

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Le mouvement de circumnutation des tiges volubiles a été attribué à une réaction géotropique latérale résultant d'un géotropisme négatif agissant selon l'axe de symétrie de la tige, tendant à la redresser, et d'un géotropisme positif, tendant à l'incliner, mais agissant sur une face latérale, droite ou gauche selon l'espèce considérée. On interprète en faveur de cette théorie le comportement des tiges volubiles lorsqu'elles s'arrêtent de nuter sur un clinostat à axe horizontal ; Teodoresco a pu cependant observer, sur le clinostat, l'enroulement de tiges volubiles autour d'un tuteur horizontal (ce qui doit permettre de nier l'influence de la pesanteur), mais ses conclusions ont elles-mêmes été discutées, et il peut être utile de les appuyer sur des données quantitatives :

1° Au cours de la révolution, la hauteur du sommet varie et présente généralement un maximum et un minimum ; la vitesse subit le plus souvent des variations parallèles à celles de la hauteur : elle est en général d'autant plus grande que le sommet est plus haut, que la tige, plus dressée, offre moins de prise à un éventuel géotropisme latéral. Sans aller jusqu'à attribuer à la pesanteur une action directe de freinage latéral, il semble au moins permis de considérer que le déplacment instantané n'est pas un effet de la pesanteur. Ces résultats ont été obtenus avec des Dioscorées, des Cuscutes, des Convolvulacées, etc. par A. Tronchet et ses collaborateurs.

2° Teodoresco n'a observé, sur le clinostat, que des mouvements d'enroulement, cas particulier des mouvements de circumnutation ; employant un clinostat incliné sur l'horizontale d'un angle a variable, nous avons étudié le phénomène plus général du mouvement libre d'exploration des tiges volubiles. Au cours du mouvement du clinostat, la plante est soumise de la part de la pesanteur à une accélération moyenne égale à $g \sin a$. La théorie du géotropisme latéral laisserait prévoir une période inversement proportionnelle à $\sin a$; nous avons observé des tiges volubiles de Cuscute et mesuré leur période (T = 23°C), en les laissant d'abord nuter autour d'un axe immobile sensiblement vertical, puis sur un clinostat oblique ; faisant ainsi varier l'action moyenne de la pesanteur entre g et $g/10$, nous n'avons pas observé de variation de la période, à 10 % près (période d'environ 90 min. chez le *Cuscuta odorata* Ruiz et Pav.). Pour $a = 0$ en revanche, par suite de la flexibilité des tiges de Cuscute non soutenues par un tuteur, la partie active de nos tiges se trouvait ramenée en dessous de l'horizontale et, dans la plupart des cas, la circumnutation s'est brusquement arrêtée ; (une tige volubile renversée ne nute pas, elle se redresse progressivement à partir de son sommet et ne reprend son mouvement que dans sa portion redressée).

Les tiges volubiles étudiées ont donc un mouvement orienté par rapport à la pesanteur, mais, ni dans sa vitesse instantanée, ni dans sa fréquence, ce mouvement ne peut être considéré comme régi par une excitation géotropique latérale. Par suite, la vitesse angulaire du déplacement du maximum d'élongation autour de l'axe de la tige, et la période selon laquelle chaque partie de la tige passe par une alternance de phases de croissance et de phases de repos apparent, sont déterminées par des facteurs internes sans relation immédiate avec la pesanteur.

CARACTERISTIQUES COMPAREES DE LA CROISSANCE ET DU MOUVEMENT REVOLUTIF DES VRILLES ET DES TIGES VOLUBILES ; INTERVENTION EVENTUELLE D'UN MECANISME HORMONAL

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Les mouvements révolutifs sont, on le sait, intimement liés à la croissance longitudinale. Pour les étudier, on effectue à intervalles de temps réguliers (généralement 5 minutes) la projection orthogonale du sommet de la partie nutante sur le plan horizontal passant par la base maintenue immobile. On relève simultanément la cote de hauteur h du sommet par rapport à ce plan. On calcule le rayon efficient R (segment rectiligne unissant le sommet à la base et variant en sens inverse de la courbure) et la vitesse moyenne Vm du sommet dans chaque intervalle de temps. La base est immobilisée dans une position telle que le mouvement peut s'effectuer librement autour d'un axe virtuel à peu près vertical.

Le mouvement révolutif des *vrilles* paraît généralement conforme au schéma suivant. La cote de hauteur h, le rayon efficient R et la vitesse moyenne Vm présentent des oscillations relativement régulières qui tendent à concorder dans le temps pour les trois grandeurs. Pour chaque tour complet ou *révolution* du sommet, h montre deux *points d'infléchissement* très marqués pour lesquels R et Vm présentent également leurs valeurs les plus faibles. Ces variations périodiques permettent de décomposer le mouvement en *demi-révolutions*. Dans chaque 1/2 révolution on distingue une *phase d'accélération* qui est en même temps une phase d'accroissement de la cote de hauteur et du rayon efficient, puis une *phase de ralentissement* caractérisée en outre par une diminution de h et de R. Notons que Vm présente continuellement des oscillations supplémentaires d'amplitude plus faible. Ce schéma général est basé sur des observations de vrilles de Cucurbitacées, Passifloracées et Légumineuses couvrant plus de 600 heures de mouvement révolutif (A. et J. TRONCHET, A. QUANTIN, L. BAILLAUD, E. CRINQUAND).

Dans la circumnutation des *tiges volubiles*, la cote de hauteur *h* du sommet présente généralement un seul minimum (*point d'infléchissement*) et un seul maximum par révolution. Le chemin parcouru entre deux minima consécutifs de *h* correspond donc typiquement à une révolution complète et non à une 1/2 révolution comme dans les vrilles. Les projections horizontales du sommet dessinent une courbe plus régulière et d'un tracé presque circulaire dont la base immobile n'occupe cependant jamais le centre. Malgré cette apparente uniformité, le mouvement révolutif des tiges volubiles ne peut pas être ramené à un schéma unique : c'est du moins ce que montrent les variations de *R* et de *Vm*. Dans le mode le plus fréquent (66 % de la durée totale des révolutions observées), les variations de *R* concordent non avec celles de sa composante verticale, c'est-à-dire la cote de hauteur comme cela a lieu pour les vrilles, mais avec celles de sa composante horizontale. Quant à la vitesse *Vm*, tout en présentant continuellement des variations de faible amplitude comme pour les vrilles, elle passe également par des phases alternantes d'augmentation et d'affaiblissement, mais celles-ci, malgré certains cas remarquables de concordance, ne présentent pas en général de relation déterminée avec les oscillations de *h* et de *R*. Ces données sont basées sur des observations de tiges de Polygonacées, Légumineuses, Convolvulacées, Apocynacées, Dioscoréacées représentant environ 1.000 heures de mouvement révolutif (A. et J. TRONCHET, L. BAILLAUD, Y. COURTOT).

Il a été démontré récemment par C. ARNAL que les *coléoptiles* présentent, eux aussi, durant leur croissance normale, un mouvement révolutif bien caractérisé. Ce fait a été négligé par les auteurs qui ont étudié le mécanisme hormonal de la croissance. ARNAL est, semble-t-il, le premier à formuler nettement l'hypothèse d'une liaison entre le mouvement révolutif des coléoptiles et le mécanisme auxinique de leur croissance. Le sommet du coléoptile de Blé produirait de l'auxine de façon périodiquement inégale sur toutes ses faces ; la circulation basipète de ces quantités inégales d'auxine engendrerait les courbures de nutation.

Cette hypothèse peut-elle être étendue au cas des vrilles et des tiges volubiles ? Certains faits connus paraissent exclure l'intervention de facteurs hormonaux circulant en direction basipète. Après décapitation des vrilles, le moignon continue à présenter des mouvements révolutifs dont l'allure générale paraît peu modifiée (WORTMANN, O. MÜLLER, H. GRADMANN). D'après L. BAILLAUD, il en serait de même, au moins dans certains cas, pour les tiges volubiles qui peuvent continuer à nuter après décapitation sans autre perturbation apparente qu'un léger redressement et un faible ralentissement du déplacement angulaire.

LES MOUVEMENTS DE NUTATION DES COLEOPTILES DE GRAMINEES

par Claude ARNAL

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Depuis DARWIN (1880), on explique la nutation par le déplacement d'une zone d'élongation maximum autour d'un organe en voie de croissance. Malgré la liaison établie depuis si longtemps entre la croissance et la nutation, nos connaissances actuelles sur le déterminisme hormonal de la croissance n'ont pas été utilisées pour tenter d'élucider le mécanisme de la nutation.

L'étude cinématographique de la croissance des coléoptiles de Graminées (ARNAL, 1953) montre que ces organes n'ont pas une croissance rectiligne, mais présentent des mouvements de nutation. Ces mouvements ont été plus spécialement étudiés dans les coléoptiles de Blé. La croissance d'un coléoptile qui se développe à la lumière et qui n'est pas étiolé se déroule de la façon suivante : jusqu'à l'âge de 50 heures environ, la croissance est très lente (sortie du coléoptile), puis la vitesse augmente régulièrement pour atteindre environ 10 microns à la minute vers la 80ᵉ heure de culture. Cette vitesse maximum reste sensiblement constante pendant une dizaine d'heures, puis diminue assez rapidement pour s'annuler vers la 110ᵉ heure. Quand la vitesse de croissance est faible, jusque vers la 65ᵉ heure, il n'y a pas de nutation. De légers mouvements de nutation apparaissent pendant que la vitesse augmente ; ils sont d'amplitude faible (environ 1 mm.) et de période irrégulière (3 à 6 heures). Mais à partir du moment où la vitesse de croissance maximum est atteinte, l'amplitude de la nutation augmente (5 à 8 mm.) et la période devient fixe (3 heures). Cette nutation régulière et de grande amplitude se poursuit jusqu'à la fin de la croissance et s'arrête seulement à ce moment.

L'observation des variations de forme d'un coléoptile pendant la nutation montre que les courbures apparaissent à 1 ou 2 mm. en dessous du sommet, puis se déplacent vers la base du coléoptile. Une courbure met environ 1 h. 30 pour atteindre la base, soit la durée d'une demi-période de nutation.

Si on décapite un coléoptile pendant sa croissance, celle-ci est fortement ralentie pendant environ 4 heures, puis, par suite de la régénération physiologique du sommet, la vitesse de croissance augmente progressivement pour atteindre, environ 15 heures après la décapitation, une vitesse sensiblement égale à celle des coléoptiles de même âge non décapités. La nutation est également arrêtée par la décapitation, mais elle reprend au moment où la vitesse de croissance atteint de nouveau son maximum, 15 heures plus tard, pour ne se terminer qu'avec la croissance elle-même. Toutefois, sur un tel coléoptile qui a été décapité, l'amplitude de la nutation reste généralement inférieure à celle des témoins.

Le déplacement basipète des zones de courbures, le fait que la décapitation arrête temporairement la nutation en même temps que la croissance, permettent de conclure que les mouvements de nutation sont régis, comme la croissance, par la circulation basipète de l'auxine. Le mouvement de nutation serait donc la conséquence d'une production périodiquement variable d'auxine dans les différents secteurs du sommet réel ou physiologique.

Mais il n'y a pas une relation directe entre la vitesse de croissance et l'amplitude de la nutation. Lorsque la vitesse augmente, pendant la croissance normale ou pendant la régénération physiologique, on sait (Went, 1942) que la quantité d'auxine augmente parallèlement ; on peut en conclure que, dans cette partie de la courbe de croissance, l'auxine est le facteur limitant de la vitesse. La nutation apparaît quand la quantité d'auxine atteint un certain seuil. Mais le fait que la nutation continue quand la vitesse de croissance diminue, à la fin de l'élongation du coléoptile, conduit à penser que, dans cette deuxième partie de la courbe, un autre facteur que l'auxine intervient dans le déterminisme de la croissance. Cependant, puisque la nutation est produite pendant toute sa durée par des variations périodiques de la production d'auxine, ce deuxième facteur doit être lui-même en rapport avec cette production d'auxine et pourrait être, par exemple, un précurseur. Dans ces conditions, on peut interpréter les mouvements de nutation comme le résultat de l'établissement d'un régime de relaxation entre deux facteurs limitants de la croissance : l'auxine et un précurseur. Ce régime apparaît seulement au moment où le précurseur devient facteur limitant de la croissance et alors que le sommet normal ou physiologique du coléoptile a acquis la possibilité de transformer en auxine une quantité plus grande de précurseur que celle qu'il reçoit.

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b) *INHIBITEURS DE CROISSANCE*

RAPPORT

GROWTH INHIBITORS

by Hans Burström
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A great number of substances are known to possess the property of inhibiting growth in a broad sense. Evenari listed several years ago some one hundred cases of natural occurrence of germination inhibitors. It is assumed that all these substances also inhibit growth in other organs, because germination can be regarded as a special case of growth only, the normal one in embryonic tissues. Among these substances are found compounds of almost every kind, inorganic and organic, simple and fairly complicated ones, from ammonia and hydrocyanic acid, over simple organic compounds such as allyl compounds and dicarboxylic acids, to lactones, essential oils, polypeptides, and alkaloids. If synthetic compounds are added, the list of growth inhibitors can be enlarged, for example, by all respiratory poisons, cytochrome inhibitors (Hackett and Schneidermann) and others. The natural growth inhibitor from beet seeds found by de Kock and Hunter can, on the other hand, according to Butler be assumed to be an inhibitor of Cu-oxidases but not of cytochromes. The explanation is obvious : growth includes a complicated and sensitive mechanism, depending upon respiration and other parts of the metabolism, and the whole system is easily deranged. Of special interest are, of course, substances which can be assumed directly or specifically to inhibit the cell elongation process proper. Two types of such inhibitors are worthy of attention as subjetcs of discussion : synthetic compounds more or less closely related to natural auxin, but acting as inhibitors, and natural compounds with an activity different from or contrary to that of auxin.

This is generally speaking the problem of anti-auxins and auxin antagonists, which plays an important role in the interpretation of the mode of action of auxin. Since these inhibitors counteract auxin, or apparently do so, they actually inhibit elongation only in shoots, but increase that in roots. The most important growth inhibitor under many conditions, for example in buds and roots, is apparently auxin itself. It is for this reason nearly impossible to denote a discussion to growth inhibitors without touching upon certain aspects of the auxin problem.

Whether a substance should be called auxin or anti-auxin, growth promotor or growth inhibitor, may wholly depend upon the test method employed ; 2, 4-D for example, is a growth promotor and supposed to exert auxin action in straight growth tests, but it is regarded by WEINTRAUB as an anti-auxin with respect to its formative effects. The definition of growth inhibitors is consequently rather arbitrary depending upon what is meant by growth.

It has been found convenient to study the effect of auxin antagonists or anti-auxins on roots, as a matter of fact, because an increase in elongation is more clearly set off from other effects, particularly non-specific toxic effects, than growth inhibitions. As far as anti-auxins are concerned such toxic effects confuse the issue especially in shoots, and they are usually not sharply defined or at all distinguished from each other experimentally.

The two-point attachment theory, advocated by HANSCH and MUIR in order to explain auxin activity, has prompted BONNER to a general formulation of the structural requirements of inhibitors of the anti-auxin type. The theory implies that an active auxin should be bound to two points on the substrate by the carboxyl and one orthoposition of the ring. A compound capable of fixation at only one point must act as an antagonist by blocking this site for the auxin. This can be brought about by : 1) substitution of both orthoposition, 2) steric hindrance in the side chain, 3) unsuitable length of the side chain, permitting only the carboxyl to be attached to the substrate. The three-point attachment theory, supported by WAIN, WEINTRAUB and others leads to about the same requirements for inhibitors and only offers a somewhat different explanation of the cause of the growth inhibiting action.

It is now of interest to see to what extent the supposed anti-auxins fulfil these requirements, and if there are exceptions to the proposed rules. As an example a selection of material has been made from an investigation by HANSEN on the influence on root growth by derivatives of acetic, α-substituted propionic and α-substituted isobutyric acids. The ring systems selected are 1-indole, 3-indole, 1-naphthoxy, 2-naphthoxy, 1-naphthyl, 2-naphthyl, phenoxy, phenyl, and cyclohexyloxy series. Of these all isobutyric acids should be strong antagonists owing to structural hindrance of the side chain, or according to the three-point attachment theory owing to lack of H in the α-position of the side chain. This also holds true, even irrespectively of substitutions of the ring. All cyclohexyloxy acids also behave according to the expectations, the orthopositions are blocked and they are anti-auxins. For the rest at least all unsubstituted acetic and α-propionic acids should generally be expected to be auxins, but there are notable exceptions. Only the 3-indole, 2-naphthoxy, and phenyl series behave wholly regularly. The 1-indolacetic acid is a weak auxin, but the corresponding α-propionic acid which ought to behave similarly, is a weak anti-auxin. The 1-naphthoxy acids are all well known as medium strong anti-auxins, which has not yet been given a convincing explanation.

It has been assumed that compounds which are weak auxins may also act as anti-auxins by replacing the more active natural auxin at its site of action. However, this sounds simple but is not easily understood. It requires the additional hypothesis that the effect does not depend only upon the affinity to the substrate, but also upon some specific activity of the acid, which has not yet been defined at all.

The most confusing conditions are found among the phenoxy acids. The propionic and isobutyric acids, which are an auxin and an anti-auxin respectively, behave regularly according to the expectations. Substitution in the 2 and 6 positions converts the former into an anti-auxin and increases this property of the latter. This is what is to be expected, since both ortho-positions are blocked. As has been stated repeatedly the acetic acid is inactive on shoots and behaves as an anti-auxin on roots, whereas the 4 or 2, 4 substituted acids are regarded as auxins. This has not been convincingly explained although THIMANN has outlined an interesting hypothesis based on the activation of the ortho-positions. Furthermore, if it is substituted in the 2 and 6 positions, it is converted into a wholly inactive compound. In this instance the substitution does not increase the anti-auxin property as with the propionic and isobutyric acids, but on the contrary annihilates it.

This inactive 2, 4, 6-substituted phenoxyacetic acid acts, however, as an antagonist against externally supplied auxin (ABERG), and it decreases also the anti-auxin action of externally supplied 2, 4, 6-substituted isobutyric acid. It has then the character of an antagonist against anti-auxin as well as against auxin. The anti-auxin effect of a 2, 4, 6-substituted α-propionic acid, on the other hand, is not influenced by simultaneously present acetic acid.

One obvious conclusion is that a distinction must be made between two types of presumed auxin-inhibitors. One type is in itself active as a growth substance, increasing growth in roots, it is represented by the isobutyric acids, and has been supposed to counteract endogenous auxin. There are on the other hand, also substances which are in themselves inactive but counteract an externally applied auxin, or both auxins and anti-auxins. Antagonists of this type can hardly act by competing with auxin at its point of action. Such a compound must, of course, also counteract native auxin, and can not antagonize anti-auxins. If the points of attachment of auxin are blocked by one antagonist its action can hardly be decreased by the addition of another antagonist competing for the same sites, because auxin is already hindered from reaching the sites. It is further pertinent to observe that externally applied auxins are more easily antagonized than the endogenous auxin. It is reasonable to assume that the antagonism takes place somewhere else, for example, during uptake or transport of the exogenous substances. We have proposed to term these substances auxin antagonists in order to separate them from the inherently physiologically active anti-auxin.

Another conclusion which can be drawn is that the superficially similar actions of the two anti-auxins, the 2, 4, 6-substituted α-propionic and α-isobutyric acids, in fact can not be identical, because they are not antagonized in the same way by the acetic acid.

The group of real anti-auxins, of course, deserves the most attention. The isobutyric acids are particularly active in this respect. Bonner has made kinetical evaluations of growth of coleoptiles in combinations of auxin and an isobutyric acid and concluded that the latter acts by competing with auxin in one enzyme system, responsible for growth. However, it is inherent in the method of kinetical evaluation of Lineweaver and Burke that it cannot conclusively prove the existence of such a competitive inhibition, it can only show if such a competition does not occur. Although very suggestive, the results of Bonner's deductions are open to criticism if other evidences point to an antagonistic action or some other kind. That auxin and isobutyric acids are not ideal antagonists has been emphasized by Rufelt. He has studied their influence on the geotropic response of roots and advocates that auxin acts on the normal positive geotropic reaction, but that isobutyric acid only regulates a subsequent very little known negative reaction. They do not counteract each other as far as geotropism is concerned, but since both geotropic responses depend upon growth, they must act on different points in the growth mechanism. This means that it is undecided whether there are growth inhibitors within the auxin group really competing with auxin at its point of action. On the other hand, it is obvious that competition between growth regulators may take place at other stages.

One point which always has to be considered in growth inhibition studies is the importance of the time factor. The following conditions may serve as an example. Phenylacetic acid and α-phenylpropionic acid are both root growth inhibitors. In tests of 24 hours' duration the acetic acid is a strong inhibitor, but after 72 hours the activity may appear to have decreased to only one tenth. On the other hand, the propionic acid starts as a very weak inhibitor, but its activity is higher in long-time experiments. Any classification of the inhibitors with regard to relative effects is wholly arbitrary. It is also well known, that roots adapt themselves to indol-acetic acid, the growth inhibitions decrease with time, and adventitious roots may be wholly insensitive to an external application of auxin ; on the other hand, the inhibiting action of 1-naphthylacetic acid may increase with time, in spite of the fact that it is the synthetic compound most resembling native auxin. It is difficult to assume that compounds with a cumulative effect and these to which the plants adapt themselves, are metabolized in the same way. — The adaptation to inhibitors may partly be due to the fact that tissues of different developmental stages are sensitive in varying degrees to growth compounds. It has been shown with several growth active compounds that cells in progress of elongation are more susceptible than these which develop in the presence of the compounds. This is an error, which has to be considered in evaluating results of growth tests of short duration. Reversibility of growth inhibitions upon removal of the inhibitor, which has been described, must also be interpreted with caution ; each cell is in a state of elongation for only a short time before it matures and is succeeded by new ones. If an inhibitor is removed from a root, the newly formed cells, of course develop normally, if the meristems have not been damaged.

These methodical errors depending upon the anatomical structure of the growing organs contribute to the confusion in the general picture of growth inhibitions by the auxin homologues. It seems permissible to conclude, however, that even within this group of closely related acids growth inhibitions of different kinds are encountered. In so far as these inhibitions depend upon a competition with, or an antagonism against the natural auxin it can be assumed that this competition can take place at different locations in the growing organ, perhaps during the formation, the transport, the uptake or at the point of action of auxin. Inhibitions by compounds closely related to auxin may also be independent of auxin.

Consequently the same may hold true of natural growth inhibitors, and we can expect to find many rather heterogenous inhibitors, even if we restrict ourselves to those which affect the cell elongation propter and even if some types of natural inhibitors show structural relations to auxin.

An apparently simple case is represented by trans-cinnamic acid. It antagonizes auxin in both shoots and roots (v. OVERBEEK), but not anti-auxins even on roots (HANSEN), and could thus be assumed to compete with auxin really at its point of action.

For the rest we know very little of the mode of action of natural growth inhibitors. Only the preparation by ROBERTS really seems to fulfil the requirement of an auxin-antagonist, because in several functions it can counteract externally applied auxin. As far as I know, it has not been chemically identified as yet. It is, on the other hand, obvious that the separations carried out by BENNETT-CLARK and KEFFORD and LEXANDER, the latter with the special aim of looking for anti-auxins, have failed to disclose substances possessing anti-auxin properties. These investigations have been restricted to acid substances, however they warrant the conclusion that growth regulators of the auxin type but of the reversed action hardly occur naturally. The substances of BENNETT-CLARK and LEXANDER affect roots and shoots in the same way, just as the inhibitor of YARDENI and EVENARI. Neutral inhibitors haye also been isolated, of which we at present know very little, for example those found by FELDMEIER and GUTTENBERG and by BONDE, and HEMBERG has traced in potatoes both an acid and a neutral sprouting inhibitor, of which the latter is the real regulator of dormancy. The chemical identification of all these unknown substances is an urgent problem.

The group which deserves the most attention is the unsaturated lactones and similar compounds, which have been isolated from different materials and apparently are widely distributed in plants. First of all are the coumarin derivatives, coumarin itself, scopoletin found by GOODWIN and TAVES, and the three coumarin derivatives isolated by BONNER and BENNETT from one and the same plant, *Thamnosia*. We have to deal with here a large group of normally occurring substances, probably specific for different plant species and all growth inhibiting in low concentrations. Systematic studies of their activity are lacking as yet, but the data at hand indicate that plant species are sensitive in varying degrees to the coumarins. This seems to be the most promising field at present for studies of specificity of growth regulators.

Little or nothing is known of how the coumarins act. POLJAKOFF-MAYBER has recently shown that coumarin inhibits hydrolyzing enzymes, for example protease in germinating seeds. The growth inhibition has also tentatively been explained by their capacity to block SH-groups by complex binding to the 3 and 4 carbon atoms. It is suggestive that ELLIOTT and LEOPOLD have found a polypeptide with the same blocking property and likewise growth inhibiting. This is also the case with chelidonic acid, which LEOPOLD and SCOTT assume to be a widespread growth inhibitor. It has further been shown by MAYER that the inhibition caused by coumarin runs parallel to the amount which cannot be recovered analytically, and it is supposed during its growth action to be either destroyed or bound to some compound, which in its turn becomes inactivated.

However, GOODWIN and TAVES here already found growth inhibitors among the coumarin derivatives with substitutions blocking the 3 or 4 positions. It has further been show by HERTZ that both a saprophytic fungus, *Aspergillus niger*, and a parasitic one, *Candida albicans*, are insensitive to coumarins even in so high a concentration as 4.10^{-4} mol. We must, however, assume that also fungi need active SH-groups in their metabolism, and consequently, coumarins can hardly produce a general inactivation of sulph-hydryl groups. It is interesting to observe, on the other hand, that fungi are equally insensitive to auxin, inhibitors of the auxin group and anti-auxins. This nearly excludes the possibility that auxins and the specific growth inhibitors interfere in some part of the fundamental metabolism common to fungi and higher plants, for example the fundamental respiratory system which has been suggested. It seems more likely that these compounds affect something specific to higher plants and particularly connected with their mode of cell elongation. There may be some intimate connexion between the activity of auxins and the coumarins, or it is simply that they both influence one growth mechanism, although independently of each other.

THE ACTION OF COUMARIN AS A GERMINATION INHIBITOR

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Between the many germination inhibitors occurring naturally in plants, coumarin is among the most potent ones and perhaps the most interesting, because of its interaction with light, temperature and certain substances.

1) *Interaction of Coumarin, Light and Temperature.*

At all temperatures coumarin depresses the germination percentage of lettuce seeds, germinating in the dark. The lower the temperature the weaker is the inhibiting action of a given concentration of coumarin. When short time illuminations with white light are used, the coumarin inhibition is counteracted by light. This counteraction is again a function of temperature and of the product of light intensity times duration of illumination (I × t). When continuous illumination is used the counteracting influence of light at a given temperature is inversely proportional to its intensity, whereas the dependance on temperature is the same as for short time illumination. The only active constituents of white light are red (6800 Å) which counteracts the inhibiting influence of coumarin and infrared (7200 Å) which enhances the coumarin inhibition.

2) *Interaction of Coumarin and other substances.*

Coumarin and Thiourea have an antagonistic effect on the germination of lettuce seed. 2,4D and coumarin a synergistic one. It is very interesting that cysteine, which alone does not effect germination at all, is a very potent inhibitor in conjunction with coumarin, and 2,4D.

By studying the effect of various metabolites in conjunction with 2,4D and coumarin it can be shown that both inhibit germination in a different way and act on different metabolic paths. For example different amino acids as Aspartic acid have nearly no effect on the inhibiting action of coumarin whereas they have a very pronounced synergistic influence in conjunction with 2,4D. Glucose-l-phosphate acted with both inhibitors as an antagonist but its antagonistic action was much more pronounced in connection with 2,4D than with coumarin. All these experiments do not throw much light on the action mechanism of coumarin. But there is one pertinent fact : Lettuce seed extracts inactivate or destroy coumarin but pretreatment of the seeds with coumarin abolishes this effect. This may mean that the inhibition caused by coumarin involves a reaction with some essential metabolite which is produced enzymatically or with the enzyme producing it.

3) *Coumarin and Enzyme systems.*

Lettuce seeds contain besides fat reserves also reserves of sucrose. This sucrose is used up by respiration during the initial stage of germination. This process is not much affected by coumarin. Fat hydrolysis is accompanied by the formation of reducing sugars. This process is blocked by coumarin. Catalase and peroxidase activities *in vitro* are not affected by coumarin, while proteolytic and lipolytic enzymes are inhibited. The germination inhibiting effect of coumarin may be explained by its inhibition of hydrolytic enzymes.

4) *The Relation between Structure of Coumarin and its Inhibiting Activity.*

A large number of coumarin derivatives was tested for their germination inhibiting activity. Any substitution in the coumarin molecule destroys partially or completely its inhibiting action on the germination of lettuce seeds. The presence of the double bond in the lactone ring and the lactone structure are essential. The fact that some derivatives (1:8 dihydrocoumarin, scopoletin, etc.) are inactive as germination inhibitors but as active as or more active than coumarin as root growth inhibitors (Goodwin and Taves 1905) shows that root growth inhibition is not necessarily the cause of the germination inhibiting action of Coumarin.

INTERACTION OF GROWTH PROMOTORS AND GROWTH INHIBITORS

by Hans Linser

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Previously reported experiments (H. Linser, *Biochim. et Biophysica Acta,* VI, 384, 1951 and X, 189, 1953) concerning the interaction of indole-3-acetic acid and some plant extracts with some synthetic and natural growth inhibitors have been continued with different synthetic growth substances. Using the paste method and measuring the growth of Avena-coleoptiles 24 hours after the treatment in % of controls (treated with pastes containing no growth regulators) two different types of growth regulators can be observed : the growth promotors (growth substances) which promote the cell elongation of intact coleoptiles in small concentrations (up to 10^{-3} m) and inhibit it when present in larger quantities ; and the growth inhibitors showing no concentration with growth promoting action but inhibiting cell elongation in the same small concentrations, which gave growth promoting action in the case of growth substances. Indole-3-acetic acid, a-naphtalene acetic acid and 2,4,5-trichlorphenoxy-acetic acid have been used as growth promoting substances and on the other hand 2,4,6-trichlorphenoxy-acetic acid, 2,3,5-trichlorthiophenoxy-acetic acid, trijodobenzoic acid and eosin as growth inhibitors. Each inhibitor has been mixed in different concentrations with different quantities of even one of the three growth promoting substances and the concentration/action curves have been drawn. The results showing a very similar type of interaction with all types of promoting and inhibiting substances indicate that the previously observed interaction between heteroauxin and eosin is not specific for the case of eosin but is the same for all other kinds of examined growth inhibitors too. It is of interest that the interaction of 2,4,5-trichlorophenoxy-acetic acid with 2,4,6-trichlorophenoxy-acetic acid differing only in the position of a single Cl-atom and of antagonistic action is of the same type as that from heteroauxin with eosin. This indicates that the formerly evaluated mathematical model of competition between promoting and inhibiting substances in filling in certain « spaces » of the living system by intermolecular forces according to a hypothesis published by the author (*Monatshefte f. Chemie*, 1954) can be applied generally for the description of the mode of action of growth substances and growth inhibitors.

INTERACTION BETWEEN AUXIN AND ANTIAUXINS AT THE LEVEL OF DEHYDROGENASE ACTIVITY « IN VIVO » AND « IN VITRO »

by Erasmo **Marrè**

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In previous researches the autor has shown that the growth inhibiting action of a glucoside, phloridzin, and of its aglycone, phloretin, is parallel to the property of these compounds to inhibit dehydrogenase activity in cell free extracts as well as in free hand sections. As phloretin is in some degree similar in structure to cynnamic acid, a natural antiauxin, the fact suggested the possibility that typical antiauxins could act on growth by inactivating some dehydrogenase system.

Researches were therefore carried out on the effect on dehydrogenase activity, either of free hand sections or of cell free extracts and mitochondrial preparations, of several antiauxins, and precisely : transcynnamic acid, diphenylacetic acid, phenylpropionic acid, dichloroanisole, coumarin and maleic hydrazide. All of these compounds, under carefully controlled experimental conditions, and at concentrations not far from those reversibly acting on growth, clearly inhibited dehydrogenase activity in the presence of various substrates. A similar inhibiting action was shown by the same compounds in regard of the capacity to reduce triphenyl-tetrazolium chloride in free hand slices from various materials.

As growth inhibition by antiauxins can at some degree be reversed by auxin a second series of experiments was undertaken to know whether indoleacetic acid could reverse also this effect of antiauxins on dehydrogenase activity. Experiments on free hand sections did show that IAA, while ineffective alone on the TTC reduction in the sections, is indeed able to reverse at a considerable degree the dehydrogenase inhibition induced by the antiauxins.

The same phenomenon was then investigated on cell free extracts and on mitochondrial preparations. Here again auxin in various experimental conditions efficiently counteracted the effect of antiauxins, thought the results appeared somewhat erratic. The attainment of more reproducible results is evidently conditioned by the identification of the enzymatic system specifically involved in the auxin-antiauxin interaction : a point presently under investigation.

The significance of these data for the problem of the mechanism of action of growth regulators is discussed.

THE INTERACTION OF ANTIAUXINS AND β-INDOLYL-ACETIC ACID IN THE GROWTH OF ROOT SECTIONS

by L. J. Audus

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The fact that the auxin β-indolylacetic acid (IAA), as well as recognised antiauxins, may stimulate root growth at appropriately low concentrations, raises the major problem of the true rôle of auxin in the control of root growth. Since antiauxins had so far been demonstrated to antagonise only the inhibiting action of high concentrations of externally applied IAA, the writer was led to propose (Audus and Shipton, 1952) a theory that IAA might not be the natural root growth hormone. It was suggested that both IAA and the antiauxins might antagonise the inhibiting action of this unknown hormone, thereby stimulating growth.

This theory, however, needed checking with concentrations of IAA causing root growth stimulation and experiments have therefore been carried out in my laboratory by N. Das using two well-established antiauxins, α-(1-naphthylmethyl-sulphide) propionic acid (NMSP) and α-(p-chlorophenoxy-)-iso-butyric acid (PCIB). Both these substances stimulate the growth of attached roots of seedlings over a wide concentrations range and antagonise the inhibition of that growth by high auxin concentrations (Aberg, 1950 ; Burström, 1950).

The technique employed 2 mm. sections excised from the extending zone of *Pisum sativum* roots. When grown in 1/2 % sucrose solutions under conditions of good aeration these may increase in length from 3 to 4 times in 48 hours. The growth rate of these sections is stimulated by one part in 10^{11}IAA. The stimulation is of the order of 15 — 30 % in the first four hours, falls to about 10 — 15 % in the next three hours and then disappears over the next twenty hours. This loss of effect may be due to inactivation of the auxin by the root sections. Concentrations above one part in 10^9 inhibit growth.

NMSP alone causes a stimulation of the growth of such sections, the degree of stimulation increasing progressively up to 30 % as the concentration increases from one part in 10^8 to one part in 10^5. At one part in 10^3 growth is inhibited. The stimulation, however, does not appear until after about seven hours and disappears again in the later stages of extension. This delay in the appearance of the stimulation may be due to a slow penetration of the antiauxin. This is borne out by the fact that the stimulation of growth by 10^{-11}IAA in the first four hours is in no way affected by a wide range of NMSP concentrations but that the smaller stimulation in the period 4 — 7 hours is antagonised. Over the period 7 — 24 hours the growth stimulations produced by all concentrations of NMSP are antagonised by IAA at concentrations of 10^{-11} and 10^{-10}.

PCIB, on the other hand, does not stimulate the growth of these sections at any concentration or in any growth stage, although inhibition appears within the first five hours with concentrations of one part in 10^5. It does however completely antagonise, even at a concentration of one part in 10^7, the early stimulation of growth by 10^{-11} IAA. Its penetration of the root must therefore be rapid. IAA at the above concentration has no effect on PCIB inhibitions.

These results cannot yet be fitted into any existing theory of the control of root growth by auxins and further experiments with other antiauxins are now in progress.

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VARIATIONS DE CROISSANCE DES RACINES ET PHENOMENES AUXINIQUES

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L'étude de la croissance des racines a fait l'objet d'un grand nombre de travaux dont les conclusions sont souvent contradictoires. Nos observations sur les racines du *Lens culinaris* Med. ont montré que, suivant leur âge, ces organes présentent des réactions physiologiques différentes, voire opposées, qui expliquent, dans une certaine mesure, les divergences rencontrées dans les publications relatives à ces questions.

Lorsqu'on examine la *croissance normale* des racines cultivées à l'obscurité, on constate que, tout au début de la germination (l'étude portant sur des plantules complètes), leur croissance est lente (Phase 1), puisqu'elle s'accélère (Phase 2) pour ralentir (Phase 3) ; c'est à ce moment qu'apparaissent les radicelles. La durée de ces phases varie naturellement suivant le matériel employé et les conditions de culture. L'étude de l'*action de la lumière* met particulièrement en évidence les phases précédentes. Ainsi, en irradiant à l'aide d'ultra-violets des racines en Ph.1, on constate que leur croissance est plus faible qu'à l'obscurité ; c'est aussi le cas, mais moins nettement, pour celles en Ph.2, tandis que des racines en Ph.3 ont leur croissance accélérée. L'étude du phototropisme montre que des racines en Ph.1 ont une réaction +, alors qu'elle est — pour celles en Ph.3. En ajoutant de l'hétéroauxine (HA) au milieu de culture, on arrive à supprimer la réaction +. Les *traitements auxiniques* des racines sont également suggestifs. Si on pulvérise de l'HA ou des substances de croissance diverses sur des racines en Ph.1, on provoque, pour une concentration pas trop élevée, une accélération de croissance, alors que des racines en phases 2 et 3 sont inhibées par le même traitement. De plus, sachant le rôle joué par la graine (cotylédons et albumen), les méristèmes et les feuilles, dans l'élaboration des auxines ou des *précurseurs* et leur activation, on peut supprimer l'apport de ces substances, en enlevant aux plantules les organes précédents. Au cours de ces expériences, on observe que les racines en Ph.1 cessent de grandir (ou du moins leur croissance ralentit considérablement), alors que celles en Ph.2 et 3 ont une croissance plus forte.

La racine passe donc par trois phases distinctes, caractérisées chacune par un « état de croissance » propre. Or, à quoi attribuer ces phénomènes, si ce n'est aux auxines mêmes contenues dans les racines. Des dosages répétés, à l'aide de diverses techniques, nous ont montré que plus la racine « vieillit », plus le taux en auxines s'élève. Dès l'instant où la vitesse de croissance commence à diminuer, on peut admettre que la teneur en auxines, qui continue à s'accroître, devient « susoptimale ». La racine se trouve alors dans un état d'inhibition dû à une concentration élevée de ses hormones et à la formation probable d'inhibiteurs. L'emploi de notre test « *Lens* » justifie cette hypothèse et montre qu'un traitement à l'HA permet de supprimer la phase d'activation, ce qui revient à « vieillir » prématurément les racines. D'ailleurs, des expériences avec l'hydrazide maléique (HM) confirment encore ce point de vue : l'HM ne modifie pas le taux en auxines des racines traitées, mais son action est plus nette (l'inhibition plus forte pour de vieilles racines) lorsqu'on ajoute, après traitement à l'HM, de l'HA.

A l'aide de ces résultats, on peut interpréter quelques-unes des observations relatives à la croissance des racines. Ces organes, en Ph.1, pauvres en auxines, auront évidemment une croissance faible, éclairée, ils grandiront moins encore (le peu d'auxines présentes étant inactivé). Un traitement à l'HA va accélérer leur développement et l'absence de précurseurs le ralentir. Le phototropisme + s'explique également, car la face éclairée, dépourvue d'auxines, grandit moins vite que l'autre. Les racines en Ph.3, trop riches en auxines, seront inhibées dans leur développement, éclairées, elles vont grandir davantage (la dose inhibitrice devient accélératrice). Un traitement par l'HA inhibe encore plus la croissance tandis que l'absence de précurseur assure son accélération. Le phototropisme s'explique encore, car cette fois la face dans l'ombre, trop riche en auxines (elle contient les auxines habituelles plus celles qui ont été déviées), est inhibée.

Ainsi, le développement des racines comprend trois étapes, placées chacune sous la dépendance des auxines dont les variations permettent d'interpréter les divers aspects de la croissance de ces organes.

THE BIOPHYSICAL ANALYSIS OF THE ACTION OF GROWTH REGULATORS

by K. Kaindl

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Linser's model idea is that molecules (effective molecules = EM) influencing cell elongation of a plant consist generally of a promoting group W and an inhibiting one H. In living structures certain places (gaps) are present into which such an EM enters either with an attaching group w activating W or with an attaching group h activating H. Naturally, in special cases, the groups W, H, w, h are not distinct groups but may be e. g. identically in pairs or zero in any combination. On principle the following scheme is possible :

1.) Promoting and inhibiting groups are present : $W_w\ H_h$, $W_w H_o$, $W_o\ H_h$, $W_o\ H_o$.

2.) Promoting group is absent : $O_w H_h$. $O_w H_o$, $O_o H_h$, $O_o H_o$

3.) Inhibiting group is absent : $W_w\ O_h$, $W_o\ O_h$, $W_w\ O_o$, $W_o\ O_o$

4.) Promoting and inhibiting group are absent : $O_w\ O_h$, $O_w\ O_o$, $O_o\ O_h$, $O_o\ O_o$

In any plant a production of EM takes place, the essential constituent of which are substances of indol-like compounds (IAA, IAN). The gaps of the living system correlating to w and h are partly occupied by the natural EM and the natural cell elongation is kept in balance by this occupation. When a complete foreign EM enters into this system this EM will attach itself to the correlating gaps following the laws of probability. But furthermore it will oust the natural EM from its place with a certain probability. Assuming, that g_o is the natural cell elongation measured e. g. by the length of an avena coleoptile and g the cell elongation induced by the foreign EM then the formula $z = \frac{g\text{-}go}{go}\ 100$ gives the percentual change of the cell elongation. Considering the four possibilities of a foreign EM (Attaching to a w-gap, ousting a natural EM from a w-gap, attaching to a h-gap, ousting a natural EM from a h-gap) the following formula obtains

$$z = A_1(1 - e^{-k_w c}) + A_2(1 - e^{-k'_w c}) - B_1(1 - e^{-k_h c}) - B_2(1 - e^{-k'_h c})$$

where the four terms mean the different possibilities stated in the brackets. A_1, A_2, B_1, B_2 denote constant values proportional to the different numbers of gaps : A_1 is prop. to the number of free w-gaps, A_2 to the number of occupied h-gaps, B_1 to the number of free h-gaps and, finally, B_2 to the number of occupied h-gaps. The factors of proportionality are measures of the efficiency of the attachments in question in regard to cell elongation. With the ousting terms (index 2) these factors are smaller than zero when the efficiency of the foreign EM is less than the efficiency of the natural EM. The values k and k' are constant correlating to the probability of attachement and ousting resp. and c is the percent concentration of the EM supplied. Empirically the following general rules for the different constant values can be given :

1.) Neither the promoting nor the inhibiting component of the natural EM can be exceeded by a foreign EM in efficiency. That means A_2, $B_2 \leqslant O$.

2.) The foreign EM always has an inhibiting component but the promoting component can degenerate to zero. That means $A_1 \geqslant O$, $B_1 \geqslant O$.

3.) The absolute efficiency of ousting can not exceed that of direct attachment. That means $|A_1| \geqslant |A_2|$, $|B_1| \geqslant |B_2|$.

4.) Excluding the natural EM a foreign EM can produce optimally an inhibition of 100 % when the concentration gets infinitely high. That means $B_1 \leqslant A_1 + A_2 - B_2 + 100$.

5.) The ousting of the natural promoting and inhibiting components resp. takes place optimally with that amount of probability by which the foreign EM attaches to the corresponding gaps. That means $\begin{matrix} k'_w \leqslant k_w \\ k'_h \leqslant k_h \end{matrix}$

6.) The inhibiting effect of the natural EM is a collateral effect and so it is to be expected that the ousting of the natural EM from the h-gaps takes place with a higher possibility than the ousting from the w-gap. That means $k'_w \leqslant k'_h$.

7.) When EM possesses a promoting component a certain probability of its efficiency is given. Quite the same goes for the inhibiting component. That means $k_w \pm 0$ except in the case $A_1 = 0$, where k_w could be zero. $k_h \pm 0$.

By means of this biophysical mechanism of action the experimental curves of action of different EM obtained by Linser's pasta-method can be interpreted and their function derived.

Promoting substances are represented by types of function for which the following conditions are valid : a) $A_1 > B_1$, b) $A_1 > 0$, $k_w > k_h$.

For inhibiting substances the function has to show : a) $A_1 \leftharpoondown 0$, $k_w \pm 0$ or $= 0$, b) $A_1 < B_1$, $k_w < k_h$.

In regard to our former symbolic sheme our rules push out group 3.) and 4.). When one checks groups 1.) and 2.) one can say :

Inhibiting substances are : $O_w H_h$, $O_o H_h$

Substances with inhibiting ousting : $O_w H_o$

The symbol $W_w H_h$ describes substances which can show promoting qualities depending on the amounts of the different constant values. All other cases of the groups 1.) and 2.) are impossible.

L'EMPLOI DES MICROORGANISMES FACILITERAIT L'ETUDE DES CAUSES DE L'INEGALE SENSIBILITE DES CELLULES A L'AUXINE ET DE SON ACTION INHIBITRICE

par F. Nysterakis

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Il est admis que l'utilisation des antagonistes de l'auxine peut nous aider à connaître le mécanisme de ses diverses actions. Toutefois, à partir des tests pris parmi les végétaux supérieurs, il n'est pas aisé de démontrer le

type d'antagonisme lorsque la dose de l'inhibiteur (substance inhibitrice ou dose élevée d'auxine) croît sensiblement et que la durée du traitement est suffisamment prolongée. Faible solubilité de certains produits, toxicité relativement élevée d'autres, ainsi que l'inégale sensibilité à l'auxine des divers tissus d'un test donné en sont les causes principales.

Les faits ci-après montrent que malgré des préjugés, en partie d'ailleurs justifiés, l'utilisation des végétaux inférieurs peut souvent nous aider à résoudre certaines questions dont l'intérêt est évident.

Des cellules situées côte à côte et, *a priori*, recevant la même dose d'auxine (cellules épidermiques et poils, par exemple), présentent des différences considérables au point de vue croissance. Ceci laisse supposer que celles qui croissent le plus synthétiseraient un ou plusieurs métabolites agissant soit en synergisme avec des doses optima d'auxine, soit comme antagonistes des doses relativement élevées.

Afin d'étudier l'action inhibitrice de l'auxine, nous avons cherché à extraire ces métabolites. Pour des raisons indiquées ailleurs (1951-54), et parce que SCHOPFER (1937), pour la première fois, avait établi que les fibres du coton cardé contiennent une substance stimulant la croissance, nous avons principalement utilisé ce matériel.

Chez *Nectria galligena*, 100 γ/l d'auxine ajoutés dans le milieu accélèrent sa croissance pondérale. Des doses $\geqslant 1$ mg/l l'inhibent. Lorsque la dose d'auxine utilisée est supprimée du milieu 5 à 30 jours après l'ensemencement de l'organisme, sa croissance normale est rétablie. La réversibilité de l'inhibition, conservée même après action prolongée de très fortes doses d'auxine (400 mg/l), se justifierait par la nature du test et du fait qu'il est constitué par des cellules d'un seul type, toutes au même titre en contact avec le milieu. L'auxine peut donc inhiber directement la croissance des quelques cellules ensemencées et de celles qui se forment par la suite. Le point de vue de THIMANN (1937), SKOOG (1939), AUDUS (1948), BERDUCOU (1950), ABERG (1950), BURSTRÖM (1950-51), etc..., se trouve ainsi confirmé.

Des extraits de fibres de coton, fournis seuls à l'organisme, accélèrent sa croissance. En présence de doses d'auxine nettement inhibitrices, ils annulent l'inhibition, que le pH du milieu soit corrigé ou non et même lorsqu'ils sont fournis au champignon 16 jours après l'auxine. De plus, entre 1 et 500 mg/l d'auxine, l'augmentation de la dose d'extrait réduit le degré de l'inhibition due à l'auxine. Pour un poids donné de filaments, un rapport plus ou moins constant entre auxine et extrait peut être démontré. Il semble donc que l'inhibiteur (auxine) et un métabolite contenu dans l'extrait entrent en compétition pour l'occupation d'un même centre actif de la cellule.

D'autres expériences nous ont prouvé que la biotine aussi annule l'inhibition provoquée par l'auxine, mais cette fois sans compétition. Rien n'est venu nous démontrer, néanmoins, que le métabolite contenu dans l'extrait soit utilisé par la cellule pour la synthèse de la biotine, bien au contraire.

Avec des extraits très actifs, il suffit souvent d'ajouter 100 fois moins d'extrait *brut* que d'auxine pour renverser l'inhibition. Dans le cas de la biotine, 100 γ/l annulent l'inhibition provoquée par 100 mg/l d'auxine.

Notons que d'autres expériences avec *N. galligena* nous ont permis de mettre en évidence un mécanisme d'action de l'effet fungicide de l'auxine en concentrations très élevées. Cet effet peut être aussi annulé à l'aide d'extrait.

Dans la plus simple hypothèse, les faits résumés montrent que, aux doses élevées d'auxine, ses molécules en excès inhibent l'utilisation d'un métabolite essentiel, distinct de ceux avec lesquels elle se combine pour stimuler la croissance. Mais, à des doses accélératrices, l'auxine agirait en synergisme avec ce métabolite.

Cette façon de voir nous permettrait de mieux saisir la signification de la courbe avec optima qui caractérise l'action de l'auxine (et peut-être d'autres substances) et de justifier les valeurs très différentes que présente la partie descendante de cette courbe, comme tout expérimentateur le sait bien.

Nous constatons que, *in vitro,* le comportement de l'organisme est influencé d'une manière subtile par le rapport quantitatif des antagonistes étudiés. Il y a lieu de supposer qu'il en est de même *in vivo* chez les végétaux supérieurs. Nous ne voulons pas dire par là que les antagonistes de l'auxine, étudiés sur *N. galligena,* contrôleraient l'action inhibitrice de l'auxine sur tous les végétaux supérieurs. Sans pouvoir exclure pour le moment une telle hypothèse, nous sommes plutôt enclins à croire le contraire.

L'utilisation de *Neurospora tetrasperma* nous a permis d'établir par ailleurs que sa croissance n'est pas inhibée par la dose considérable de 250 mg/l d'auxine et que dans certains milieux peu favorables à sa croissance, 100 mg favorisent son développement. L'extraordinaire résistance de cet organisme pourrait se justifier en admettant qu'il synthétise en excès le métabolite avec lequel l'auxine aurait pu interférer (ce que nous cherchons à établir), ou encore que ni l'utilisation ni la synthèse d'un métabolite essentiel ne sont perturbées par les molécules d'auxine en excès.

En définitive, les Végétaux supérieurs, ou leurs parties, ne semblent pas constituer des tests de choix pour exploiter le concept métabolites antagonistes. Par contre, l'utilisation des Végétaux inférieurs s'avérerait profitable pour aborder, au moins dans une première étape, certains problèmes fondamentaux de la physiologie moderne.

Il serait préférable, bien entendu, d'expérimenter directement sur les Végétaux supérieurs. Mais il y a lieu d'éprouver, croyons-nous, les mêmes appréhensions que les éminents devanciers de BEADLE et TATUM, SCHOPFER, WOODS, cherchant à établir l'élaboration de structures plus compliquées à partir des molécules simples, etc..., en se servant uniquement des animaux supérieurs. C'est la raison pour laquelle nous désirons rendre publiques ces quelques réflexions.

THE PHYSIOLOGY AND CHEMISTRY OF 2,4-DICHLOROPHENOXYACETIC ACID ACTION ON RESISTANT AND NON-RESISTANT PLANTS

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The development of selective herbicidal agents has been extremely rapid in the last ten years, but the elucidation of the mechanism of action has progressed slowly. In an attempt to study in detail the absorption, distribution and chemical modification of one of these compounds, we initiated a series of experiments in 1948 dealing with the action of 2,4-dichlorophenoxyacetic (2,4-D).

In order to follow minute quantities of 2,4-D in plants, the compound was synthesized with carbon-14 in the carboxyl group. This permitted us to apply quantities of the herbicide which had pronounced physiological activity but did not kill the more sensitive plants.

Seedlings of *Phaseolus vulgaris* L, var. Red Kidney were grown in nutrient culture under conditions of constant temperature, humidity and light. At the age of 9 days, a droplet containing 10 micrograms of radioactive 2,4-D was applied to one primary leaf of each of a series of plants. At three different intervals after treatment, a group of the seedlings was harvested, divided into 10 fractions and dried over P_2O_5 in a vacuum desiccator, then each fraction was oxidized by the Van Slyke-Folch wet combustion technique and the carbon converted to barium carbonate. Radioactivity counts on samples of barium carbonate from each plant part provided a measure of the distribution of carbon-14 in the plant.

Similar experiments with *Avena sativa* L., var. Victory, have been completed. To bring about a physiological response as severe as that observed in beans, the dosage had to be increased fivefold- to 50 micrograms per plant. On the basis of comparable dry weight, the oat plants received 50 to 100 times the amount of 2,4-D per milligram dry weight of the plants at time of harvest.

In comparing the resistant (oats) and non-resistant (beans) plants, a marked difference in the percentage of applied 2,4-D which was absorbed is apparent. With bean plants the amount of radioactivity found in the tissue varied from 25 to 60 % of that applied to the treated leaf. With oat seedlings the percentage ranged from 0.33 % to 1.66 %. Thus one may conclude that a partial explanation for the difference between sensitive and resistant plants may be based on absorption of externally applied herbicides. However, in these experiments the total amount of radioactivity in the oat seedlings was of the same order or greater than that found in the bean seedlings. The pattern of distribution was similar in the monocot and the dicot. It became necessary, therefore, to seek some explanation other than absorption or distribution to account for the differences in response to 2,4-D between resistant and the sensitive species of plants.

Perhaps the answer may be found in the manner in which 2,4-D combines with constituents of the plant. A procedure for fractionating the constituents of treated plants has been developed which permits the isolation of nearly all of the radioactive materials. The results of this analysis of bean and oat seedlings will be presented and the relationship to mechanism of action will be discussed.

c) *MECANISME DE CROISSANCE PATHOLOGIQUE*

NOUVELLES OBSERVATIONS SUR L'ACTION D'UNE HORMONE SYNTHETIQUE

par Paolo Meletti

Istituto Botanico della Universita, Cagliari

La présente recherche a été faite dans le but d'étudier les réactions histologiques produites par l'acide 2,4-dichlorophénoxiacétique (2,4-D), hormone synthétique bien connue pour ses larges applications, lorsqu'elle est administrée à doses contrôlées.

Nous avons fait par conséquent des injections de 2,4-D en solution aqueuse (eau de source) à des branches de *Cereus validus* Haw. détachées de la plante mère et placées dans des vases avec terre, mis en serre, à une température de 18-20° C. Le choix du témoin biologique employé est lié à la particulière facilité avec laquelle les tissus de *Cereus validus* reçoivent les injections faites avec une seringue commune, quelle que soit la dose ou la région dans laquelle on fait l'application. Dans chaque cas nous avons préféré limiter les traitements aux seules côtes, car en elles on peut fixer, avec une bonne approximation, les marges de l'aire de diffusion du liquide : cela acquiert une particulière importance pour le *Cereus*, vu que chez lui, en général, sont absents les phénomènes de translocation du 24-D, comme le démontre le constant retour de réactions locales.

Les injections ont été faites avec une aiguille n° 12, de 3 cm de longueur, orientée parallèlement au long axe de l'organe, pénétrant à l'extrémité supérieure de la côte, de façon qu'elle vienne à se trouver dans une position intermédiaire entre le bord externe et la partie basale interne de la côte même ; de cette façon la pointe de l'aiguille est située au centre de la zone de diffusion du liquide (« zone D ») qui couvre, dans le sens longitudinal, une aire à peu près circulaire d'une superficie d'environ 5 cm^2, tandis que, en sens transversal, elle intéresse toute l'épaisseur de la côte. Nous avons fait l'injection de 2 cc. pour chaque côte, arrivant à un maximum de 6 injections par plante, nombre moyen des côtes du *Cereus*. Les 4 premières applications ont été faites toutes les 48 heures, les suivantes à distance de 8 jours l'une de l'autre.

Dans un premier lot d'expériences on a essayé 5 concentrations de 2,4 dans l'eau (10, 20, 50, 100 et 200 mg/l), dans le but de fixer les limites d'efficacité du 2,4-D même appliqué au *Cereus* : nous avons ainsi pu établir que les concentrations 10 et 20 mg/l sont inactives, les concentrations 50 et 100 mg/l favorisent le développement de tumeurs, la concentration 200 mg/l induit des phénomènes de toxicité, marqués essentiellement par la constitution d'aires déprimées, correspondante à la zone D, sur les deux côtés des côtes traitées.

La connaissance de ces données nous a conduit à effectuer un second lot d'expériences avec la concentration 100 mg/l, répondant mieux aux effets de l'induction des tumeurs, en absence de phénomènes toxiques.

Les formations de tumeurs déterminent une augmentation maximum d'environ 3 fois du diamètre de la côte normale et sont connexes à des phénomènes d'hyperplasie et hypertrophie des tissus profonds, lesquels poussent vers l'extérieur les éléments de structure plus périphériques. Le noyau central de la tumeur occupe une aire correspondant à peu près à la zone D : naturellement, dans beaucoup de cas, le passage aux structures normales est graduel. Le développement de la tumeur est lent, et il est difficile d'établir quand il commence, spécialement lorsque l'on veut se fier aux seules observations macroscopiques : en tous cas, à la suite des contrôles faits, nous sommes amenés à penser que la réaction des tissus suit, à brève échéance, l'application de l'hormone. Dans les essais faits, précisément à une température de 18-20° C., vingt jours après le début du traitement, nous avons déjà une augmentation du diamètre de la côte de 1,5 fois, tandis que le développement maximum de la tumeur est atteint après cinquante jours environ.

En général les réactions obtenues pendant le cours de la présente recherche rentrent parfaitement dans le cadre d'action du 2,4-D, même si pour le *Cereus* on a le déplacement des valeurs de concentration qui induisent les différents effets, Traitements d'application facile donnent des réactions constantes, s'il est vrai que l'application de 2 cc. de solution aqueuse de 2,4-D 100 mg/l, donne lieu, en tous cas, au développement de tumeurs, autant qu'il y a de côtes traitées ou injections faites. En outre on a toujours et seulement des réactions locales, ce qui permet en outre d'utiliser à nouveau le matériel traité, avec la répétition des traitements à divers niveaux à des branches entières ou décapitées.

L'absence de phénomènes toxiques, dans le matériel traité précisément avec la concentration 100 mg/l, permet de suivre, pour une longue période, l'évolution des phénomènes d'hypertrophie et d'hyperplasie : nous avons ainsi pu observer comme les formations de tumeurs se résolvent souvent par la constitution de racines adventives, par différenciation des tissus de formation récente, qui dérivent de la stimulation à la division des cellules des tissus profonds (hyperplasie).

Assez longue est la période de temps nécessaire pour que le développement maximum des tumeurs puisse se réaliser ; de toute façon, se basant sur les précédentes expériences, nous avons des raisons de croire que la rapidité de réaction puisse être augmentée en effectuant les traitements en conditions de température plus élevée.

PRESENCE ET ROLE DE L'AUXINE CHEZ LES PLANTES VIROSEES

par Jean Pavillard
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Went (1951) a montré qu'on peut expliquer certains symptômes des viroses en termes d'*auxine.* Il a également pensé pouvoir tirer de ces maladies des exemples démontrant l'existence des différents facteurs qui, d'après lui, interviennent dans le développement des tiges et des feuilles.

1. — Dans un travail consacré à l'étude de la croissance des plantes virosées, j'ai vérifié que chez ces plantes l'auxine n'atteint pas la concentration observée chez les plantes normales : cas de la Pomme de terre, de la Tomate. Mais sa répartition n'est pas modifiée par la présence de virus : le *gradient décroissant d'auxine* existe parfaitement net dans les Tomates inoculées par le virus de la Mosaïque du Tabac, sauf au cours de l'*incubation,* où les perturbations sont liées au mode d'envahissement des feuilles par le virus. Ceci explique que ni la Tomate ni le Tabac ne présentent, sous l'action de la Mosaïque du Tabac, de développement anormal des bourgeons axillaires dormants.

2. — La « *constitution auxinique* » des organes n'est pas modifiée. Le rapport $\frac{\text{auxine libre}}{\text{auxine liée}}$ a la même valeur pour des feuilles de même rang aussi bien chez les Tomates virosées que chez leurs témoins. Dans les deux lots, la libération enzymatique de l'auxine, à partir de la fraction liée, suit la même loi.

3. — Dans la zone du bourgeon terminal, les variations de la teneur en auxine rendent compte avec la plus grande précision des variations du rythme de croissance des plantes virosées pendant les premières semaines de la maladie. L'auxine commande réellement la croissance dans cette zone.

4. — Plus les feuilles de Tomate sont déformées sous l'action de la maladie, moins elles contiennent d'auxine (par g. de limbe, ou par unité de surface). Malgré cela, la diminution de teneur en auxine n'a pas d'influence sur le *développement* des plantes : nombre de feuilles formées, et poussée des racines adventives de boutures prélevées sur des plantes extrêmement affectées par la maladie.

5. — Le même virus peut agir de façons très différentes sur la même plante, suivant l'époque de l'année, et les conditions d'environnement. Il faut en conclure que le virus de la Mozaïque du Tabac, en dehors de son effet sur la teneur en auxine, agit également sur d'autres facteurs, en par-

ticulier sur un facteur de croissance foliaire analogue à la *Phyllocaline* de WENT.

6. — Il paraît donc bien possible d'expliquer *certains aspects de la croissance des plantes virosées d'après les modifications de la teneur en auxine* de ces plantes, mais, en ce qui concerne *leur développement*, on doit faire appel à d'autres facteurs. Suivant la nature chimique du virus en cause, les modifications seront de tel ou de tel type, mais l'action des facteurs externes peut amener des variations profondes dans les effets morphogènes d'un même virus.

7. — Il reste à expliquer comment des maladies à virus, qui sont de type hypoauxinique, peuvent aboutir aux mêmes morphoses tératologiques que des *doses trop fortes* d'auxines apportées de l'extérieur sur les bourgeons terminaux et les organes jeunes : action du 2-4-D. sur les feuilles de Tomate (cf. ZIMMERMAN 1951).

La comparaison des mécanismes physiologiques intéressés par l'action des virus et de ceux qui président à l'instauration d'un état de carence (SKOOG 1940) peuvent probablement permettre de comprendre les mécanismes d'action des auxines.

COMMUNICATIONS DIVERSES

TRACER STUDIES IN CASTOR BEAN PLANTS WITH 2,4-D-1-C^{14}

by Roger M. Blouch

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These studies were undertaken to determine effects of soil moisture, temperature, concentration of 2,4-D, and phloem blockage on the entrance, translocation, accumulation and metabolism of 2,4-D-1-C^{14} in castor bean plants.

In one test series, 5, 10 and 20 lambda aliquots of 1000 ppm 2,4-D-1-C^{14} were placed on fully expanded leaves of rapidly-growing normal plants and slowgrowing, low temperature-stunted plants. The total amount per plant was placed within a lanolin ring, on the midrib near the base of the petiole. Later observation of exposed films revealed that only a small portion of the 2,4-D actually entered the plant, although 5 lamba aliquots produced radioautograms of sufficient density.

Comparisons revealed that 2,4-D penetrated the leaf and was translocated about as well in the rapidly-growing plants as in the temperature-stunted plants. With time, however, radioactivity declined rapidly in the vigorous plants while persisting for long periods in the stunded plants.

In another test series, 5 lambda aliquots were applied on rapidly-growing normal plants and slow-growing drough-stunted plants. In both types of plants radioactivity appeared in the root systems within 4 hours. Between 4 and 24 hours all radioactivity disappeared in the roots of rapidly-growing plants, but persisted for at least 5 days in the roots of the drouth-stunted plants.

The results of these tracer studies with 2,4-D-1-C^{14} indicate that herbicidal efficacy is not necessarily due to differences in cuticular composition or translocability with in the plant. On the contrary, field and laboratory experience combine to suggest that the rate of metabolism of the plant is of much greater importance, since penetration and translocation were very nearly the same in both normal and growth-retarded castor bean plants. The entry of 2,4-D into metabolism is apparently necessary for herbicidal action, possibly as a competitive metabolite in the auxin-enzyme complex.

Steam-ringing of castor bean plants treated with 2,4-D-1-C^{14} substantiated reports of other workers who suggest that 2,4-D moves with sucrose in the phloem stream. Large accumulations of C^{14} appeared in the swelling above the rings.

THE DEVELOPMENT OF SPONTANEOUS AND INDUCED CURVATURES IN ROOTS ROTATING ON A KLINOSTAT

by Poul Larsen
Universiteter i Bergen

Seedlings were rotated parallel to the horizontal axis of the klinostat. Their roots developed irregular, spontaneous curvatures at one revolution per 0.25 - 2 min., but remained approximately straight at one rev. per 16 - 64 min. If roots were geotropically stimulated and then rotated at one rev. per 0.5 - 2 min., the resulting geotropic curvatures increased continuously for at least 19 hours, whereas at one rev. per 16 and 32 min., roots straightened out again. Since the effect of rotation on the development of both spontaneous and induced curvatures thus depends on the velocity of the rotation, it is concluded that the magnitude of this effect is determined by the magnitude of the individual geotropic stimuli, active during one half revolution, and not by their sum, which is independent of rotation velocity. At higher rates of rotation the individual stimuli are too small to correct spontaneous curvatures. At decreasing rates of rotation, the direction of growth of the root is actively controlled by the increasing, omnilateral geotropic stimuli.

The « autotropic » straightening of a geotropic curvature in a rotating root, is not caused by an inherent tendency of the root to become straight, but is induced by the individual, geotropic stimuli, active during one half revolution. If these stimuli are too small, the root continues to curve in the originally induced direction.

The geotropic presentation time, determined after rotation on the klinostat, is shorter, the higher the rate of rotation.

MODIFICATED TULIP FLOWERS DURING AT LEAST TWO YEARS, ON ACCOUNT OF RADIATION WITH FAST NEUTRONS

by de Mol
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Bulbs of Parrot Tulip *Texas Gold* have been radiated in 1951 with fast neutrons (doses 60 r and 100 r ; size of the bulbs 9 cm and 12 cm in extent).

As well on the mainbud — which is developing on base of the endbud and flowering a year afterwards (1954) — as on the endbud (flowering 1953) it appeared, that the most strongly radiated bulbs has suffered most, and the small more than the large ones.

On weight determination of the earned bulbs this clearly appeared. On a second trial (a year afterwards, exclusively 9 cm bulbs, doses 60 r and 90 r, considerably higher gamma-raying) the condition of the plants was so bad, that all of them perished.

On the radiation experiments of 1951 there is question of modification during two sequent years. The metabolism of all treated plants have had a bad process. As well the anabolism as the katabolism remained beneath the normal level. Trials with slow neutrons did not produce any or none modification ; rather mutation.

From Darwin *Utopia* namely arose a larger shape, called :

Irène Joliot-Curie. I am growing now hundreds of samples of it. The treatment with fast neutrons has been considerably more injurious to the growth than roentgen radiation (doses 500 r and 600 r).

On mentioning doses of 60 r and 100 r for the radiation with fast neutrons, it is to be observed, that damaging by fast neutrons has been considerably more important than that by the gamma-rays in the cyclotron, even 10 times larger.

EVOLUTION DES CARACTERES MORPHOLOGIQUES ET ANATOMIQUES DANS DES CULTURES DE TISSUS VEGETAUX

par Pierre Nobécourt

Faculté des Sciences de Grenoble

et Geneviève Hustache

Ecole Française de Papeterie (Grenoble)

Des cultures de tissus végétaux, entretenues depuis de nombreuses années, ont présenté des modifications d'aspect extérieur ou de structure.

On distingue maintenant dans notre souche primitive de tissus de Carotte, datant de 1937, 3 variétés dont les caractères se conservent au cours des repiquages successifs ; l'une, de consistance dure, produit parfois des racines ; une autre, molle, d'un vert jaunâtre, ne produit jamais de racines ; enfin, une troisième, compacte, d'un vert clair, ne forme également jamais de racines.

Dans une souche, datant de 1944, il existe 2 variétés extrêmement différentes : l'une, formée de tissus mamelonnés, verts, ne produisant pas de racines ; l'autre, brunâtre, donnant de très nombreuses racines et, parfois, de petites feuilles.

Nos cultures de tissus de Rosier, proliférant depuis 1944, présentent aussi maintenant diverses variétés, à caractères transmissibles. Certaines variétés ont une consistance ferme, et sont globuleuses, hémisphériques ou s'étalent sur la gélose ; d'autres sont extrêmement molles et fragiles et deviennent très volumineuses. Ces cultures ont, en général, une teinte blanchâtre ou crème, mais nous avons isolé une variété qui verdit pendant la belle saison.

Au point de vue anatomique, les cultures de tissus de Carotte ont conservé la propriété de former des cellules lignifiées ; dans les tissus de Rosier, certaines lignées ont perdu cette propriété.

Il est possible que les variations constatées dans les tissus de Rosier résultent de la présence ou de l'absence de lumière, ou de la dose d'acide indol-acétique contenue dans le milieu ; dans les tissus de Carotte, ces causes ne peuvent être invoquées, puisque les cultures observées ont été constamment faites à la lumière et sur milieu contenant toujours la même quantité d'acide indol-acétique.

RECHERCHES SUR LA CROISSANCE ET LA FORMATION DE TUMEURS OU DE RACINES PAR DES FRAGMENTS D'ORGANES VEGETAUX CULTIVES DANS L'EAU

par Jean **Politis**

Professeur de Botanique à l'Université d'Athènes

Au cours de nos recherches nous avons observé que des fragments d'organes végétaux peuvent se conserver vivants, dans l'eau renouvelée deux fois par jour, pendant quelques mois ; ils présentent alors une multiplication cellulaire intéressante. Cette méthode de culture dans l'eau, nous l'avons appliquée à des organes de différentes plantes ; nous exposons dans la présente note les résultats de nos expériences sur quelques espèces des genres *Brassica* et *Raphanus*.

Brassica oleracea capitata. — Nous avons pris pour nos expériences des individus de cette plante que nous nous sommes procurés fraîchement coupés. Chaque individu a été placé dans un récipient en verre contenant de l'eau et renversé de manière à ce que la tête (pommes de feuilles) soit immergée dans l'eau, et le bas de la tige dans l'air. Après quelques semaines nous avons observé que les cellules de la moelle, qui se trouvaient sous la surface traumatique de la tige coupée, commençaient à se multiplier. De cette multiplication se formaient des racines placées habituellement en cercle. Cette provenance de racines à partir de la moelle s'observe pour la première fois. Ces racines tant qu'elles se trouvent dans l'air restent très courtes ; mais, dès qu'on les met au contact de l'eau en renversant la plante, elles commencent à s'accroître vite en longueur. Outre des racines, il se forme, à partir des mêmes cellules, un cal de cicatrisation et des tumeurs d'une structure anatomique particulière, à cellules très longues, différant des autres cellules de la moelle.

Brassica rapa v. *rapifera* et *Raphanus sativus.* — Des résultats analogues ont été obtenus en coupant transversalement la racine charnue. En outre la pointe de la racine étant immergée dans l'eau de manière à maintenir la surface de section émergée, cette surface a produit des racines. D'autre part la culture dans l'eau de fragments de racines d'autres plantes (Betteraves, Carottes, etc.) a donné lieu à la formation de tumeurs.

PHYSIOLOGIE COMPAREE DE LA THERMOGENESE CHEZ DIVERSES GERMINATIONS

par H. Prat
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Au moyen d'un micro-calorimètre Tian Calvet, il devient possible d'enregistrer d'une façon continue les variations de la thermogenèse au cours de la germination d'une graine. Nous avons enregistré les thermogrammes d'une trentaine d'espèces, à des températures comprises entre 17 et 38° C. Dans des conditions définies de température et d'humidité la forme de la courbe et la grandeur de ses caractéristiques principales : durées de la thermogenèse physico-chimique et du temps mort, débits thermiques au maximum de la première phase et au bout de 24 ou 48 h., varient d'une espèce à l'autre et peuvent être employées comme caractères spécifiques. Elles ne sont pas aussi strictement liées qu'on pourrait le croire à la taille de la graine. Dans l'intervalle de température mentionné, une élévation de température produit un raccourcissement de la durée de la thermogenèse physico-chimique et du temps mort. Une déshydratation préalable des graines augmente la valeur de la thermogenèse physico-chimique. A la condition de ne pas être trop poussée (la perte d'eau ne doit pas dépasser 5 % du poids de la graine sèche chez les céréales) cette déshydratation préalable accroît aussi le débit de la thermogenèse biologique, mesurée au bout de 24 ou 48 heures.

ON THE EFFECT OF MUSICAL SOUND OF VIOLIN ON THE GROWTH OF *MIMOSA PUDICA* L.

by T. C. N. Singh and Stella Ponniah
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Pfeffer in his classical work « *The Physiology of Plants* » has made a casual reference that an attempt to stimulate the stamens of Cynareae by sound waves was without success. It has, however, recently been shown by Singh and Ponniah (1) that sound waves (2) do positively affect the protoplasmic streaming in the cells of *Hydrilla verticillata* Presl. Sound produced by violin was found to be most potent in bringing about the effect. Encouraged by this result an investigation was undertaken on the effect of *Maya-Malva-Gaula Raga* (a morning *Indian Karnatic* tune) played on violin at a pitch of 5, on the growth of *Mimosa pudica* L.

Seedlings of the same age and vigour were transplanted in pots. When the plants had been well established, they were divided into two lots, namely *control* and *experimental*. The *experimental* lot of plants was excited every day for a period of 25 minutes (1) between the hours of 6 A.M. and 7 A.M. by playing the *Maya-Malva-Gaula Raga* on violin at a pitch of 5 in a closed room provided with normal ventilation.

Care was taken to hoe all the pots equally at proper intervals, and to irrigate them with equal volumes of water. The soil in all the pots was of the same constitution. No manure or fertilizer was added to any of them.

After excitation of the plants, the *experimental* pots were kept day and night in close proximity of the *control* plants in the open. Observations were taken every week.

For the sake of convenience the experimental results may briefly be summarized under three heads : (a) Prickles, (b) *Branches* and (c) *Leaves :*

(a) *Prickles :* From a close computation of the data in respect of prickles, it was found that the number of prickles in the experimental plants was about 45 % higher than the control.

(b) *Branches :* The production of branches on the experimental plants was more profuse than the control. On computation of the total length of all these branches it was found that the percentage of total growth in length of the experimental plants was as high as 50 over the control.

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(1) On the effect of sound on protoplasmic streaming in the cells of *Hydrilla verticillata* Presl. *Proc. Indian Science Congress* (*Lucknow*), Part III, p. 119, 1953.

(2) Produced by tuning-fork, flute, *sruti*-box, *veena* and violin.

(1) This period of 25 minutes for exciting *M. pudica* plants was fixed from the experience derived from our experiments cited above.

(c) *Leaves :* Also offer very consistent results, namely, the number of leaves after the zero day of excitation has been increasing more rapidly in the experimental plants than the control.

This experiment has been extended to other plants also like *Desmodium gyrans* DC., *Impatiens balsamina* L. and *Tagetes erecta* L. with positive results, namely the growth in height and size of leaves of the experimental plants have been found to be decidedly greater than the control.

THE SIMULTANEOUS INFLUENCE ON THE GROWTH OF Hg SALT SOLUTIONS AND Pb PLATES AT A SHORT DISTANCE OF THE PLANT

by Vincenzo Rivera

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A remarkable biological effect on growth is evidenced in the *Lupinus albus* when subjected to a joint action by metal salt solutions in the ground and lead plates placed at a short distance and acting via the air : the biological action of metals at a distance as affecting superior vegetables was discovered and illustrated by me since 1930 in connection with research studies carried out in the field of the biological action of penetrating radiation (a brisker growth was noticed as the consequence of the elimination of such radiation by a 15 meter thick body of water).

The germination of seeds of *Lupinus albus* in present day tests was carried out in sand and in 4 to 0,5 % $HgCl_2$ solutions, the distance of the seeds from the plates varying from 1 to 6 cm.

A marked biological effect was obtained in *Lupinus* by sowing seed on a ground which had been wet with 1 % mercury chloride ten days before ; the germination and development of buds were protected by a lead screen in one case and by cellophane and cardboard in another. In both cases the screens were removed one week after the germination of seeds had taken place.

The *depressing action of mercury bichloride* applied to the roots, which had been evidenced in control cultures under a cellophane and cardboard screen, *was offset* by a one or three-plate lead screening. The germination of seeds was equivalent at the beginning, but further development stages have revealed marked differences in the growth of aerial and root buds.

Other tests, carried out by spraying sand cultures of *Lupinus albus* with a very strong dose of $HgCl_2$ (4 gm. per 100 cc of H_2O) showed that the buds screened by cellophane and cardboard were sick and incapable of producing small stems, whereas the seeds protected by a lead screen (at a 6 cm. initial distance) succeeded in emitting aerial buds which were capable of growing up for 14 days, though showing signs of uneasiness on the 13th day.

The above tests give further stress to the *correcting* action which the exciting influence of a *lead* plate exerts — whenever placed at a distance from vegetables — against the paralysing action of strong $Hg\ Cl_2$ concentrations.

III. Physiologie écologique et écologie expérimentale

1. *L'EAU DU SOL — SON ABSORPTION RESISTANCE A LA SECHERESSE*

L'EAU DU SOL — SON ABSORPTION

RAPPORT

SOME FACTORS AFFECTING ABSORPTION OF MOISTURE BY PLANT ROOTS

by A. H. Hendrickson and F. J. Veihmeyer
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The factors affecting absorption of moisture by plant roots are many and varied, but, because of space limitations, only a few will be discussed in this paper. Chief consideration will be given to the soil and to soil moisture conditions.

Soil is composed of particles of various shapes and sizes touching each other at certain places. The spaces not occupied by soil particles contain water and air in various proportions. The spaces between the solid particles are filled with water at saturation, but this condition seldom, if ever, occurs in the field. When drainage is complete, they contain some water and some air. Later, if plants are growing in the soil, the proportion of water decreases and the amount of air increases. Addition of water to the soil by rain or irrigation increases the amount of water and decreases the air.

Movement of Water in the Soil.

Movement of water in the soil takes place principally by gravity, and the movement is mostly downward except in the presence of impervious layers, where lateral movement takes place. In irrigated areas the downward movement is increased by the downward pressure of the standing water on the surface of the soil. Where drainage is not impeded, rapid downward movement of water following a rain or irrigation may be considered complete in two or three days. This soil moisture condition is known as the field capacity, and has a constant value for a given soil. In the absence of growing plants, very little additional movement of moisture takes place, except in the top six or eight inches which dries by evaporation from the surface. Movement by capillarity or by water in the vapor phase is too slow and too small in amount to satisfy the transpiration needs of plants.

Plants obtain water for their needs by the extension of the roots into moist soil, and by the exploration of the soil both laterally and vertically. The volume of soil explored may be restricted by impermeable layers or by the inherited characteristics of the plants. Plants that are naturally shallow rooted make use of a smaller volume of soil than those with more extensive root systems. Other plants that have sparse root systems, may extend their roots to considerable depths, but they may not occupy all of the soil. Plants with extensive root systems may be widely spaced in areas of low rainfall, thus providing a large volume of soil from which the moisture can be obtained. While plants are able to extend their roots into moist soil, they are unable to do so in dry soil. Plant roots obtain water by extending into and absorbing it from moist soil, not by the water moving toward the roots.

Force With Which Water is Held in the Soil.

The energy required to remove one gram of water at the field capacity is of the order of 0.3×10^6 ergs. The energy required is not appreciably increased until the moisture condition, known as the permanent wilting percentage, is reached, when about 16×16^6 ergs are required to remove one gram of water. At the permanent wilting percentage, the water is held so tightly that the plant is unable to secure sufficient water for its needs, and wilting results. Most observers agree that wilting is a sudden natural phenomenon of plants when the soil moisture content reaches a certain level. Plants that are turgid one day may be wilted the next. Various physical measurements have also shown a sudden and large increase in the force with which the soil particles hold the moisture at the permanent wilting percentage.

The permanent wilting percentage is a constant for a given soil. Experiments with plants in containers have shown successive wiltings at the same moisture content. Likewise, orchard trees, on a reasonably uniform soil, have reduced the soil moisture to the same content during the rainless summers of California, year after year. All plants seem to have the ability to reduce the soil moisture to the same content. This concept was first brought out by BRIGGS and SHANTZ in 1912, who showed that widely different plants on a given soil wilted at the same soil moisture content.

Concept of Readily Available Moisture.

Plants function normally between the field capacity and the permanent wilting percentage. From this evidence, we may reason that soil moisture is readily available between these limits.

It is sometimes held that because the tightness with which water is held by the soil increases as the soil-moisture content decreases, transpiration and growth likewise must decrease. This, however, is not necessarily so when the soil-water-plant system as a whole is considered. The total free energy of the soil moisture surrounding the roots at the permanent wilting percentage is -0.16×10^8 ergs per gram. That of the air at 40 % relative humidity and 30°C is -12.8×10^8 ergs per gram giving a decrease in free energy from soil moisture to the air of -12.6×10^8 ergs per gram when the plant is under these specified conditions. If this decrease occurred through a tree 10 meters high, there would be an average force tending to pull water up the tree of 12.6×10^5 dynes per gram, a force approximating 1300 times gravity.

The cause of failure of the plant to obtain enough water from the soil at the permanent wilting percentage may be explained by the position of the permanent wilting percentage on the energy soil-moisture curve, in the region where a slight decrease in moisture content results in a great increase in resistance to removal of the water.

Drouth Resistance.

It is a well known observation that certain plants are able to survive in dry locations while others are not. Formerly, it was thought that some plants were able to secure more water from the soil than others, and thus survive. Inasmuch as it has been shown that all plants reduce soil moisture of a given soil to the same extent, this explanation of drouth resistance is no longer acceptable. The ability to survive during long periods of drouth is due to inherent characteristics of the plant itself, in that it does not die and is able to resume growth when moisture is again available.

Factors Affecting Absorption.

Since water is readily available between the limits previously described and does not affect absorption, we may turn our attention to other factors affecting absorption both as to amounts and rates. One method of doing this is by systematic soil sampling and by observation of other factors which might influence the process. After the determination of field capacity and the permanent wilting percentage of the soil in a given experimental area, soil sampling at frequent intervals will provide information about the changing soil-moisture contents from which may be obtained the amounts and rates of loss. This work, of course, may best be carried out in areas where summer rains do not complicate the situation.

With permanently rooted crops such as orchard trees, forests, alfalfa and pastures, absorption is markedly influenced by climatic conditions. Little or no absorption takes place when the plants are bare or in cold weather. Absorption begins in spring and increases with temperature and increase in leaf area. Periods of cool, cloudy weather reduce absorption. If the permanent wilting percentage is reached, absorption is reduced to a very small value. Systematic soil sampling permits the expression of absorption either as a percentage of moisture on the basis of weight of dry soil or on a volumetric basis. Experiments in California have shown that use of water (absorption by roots) is usually greatest in June, July, and August. Absorption is correspondingly less in the spring and in the fall, when the days are shorter and frequently cooler and cloudier than in the summer.

Sometimes in dense soils, absorption of moisture is greatly reduced. Roots are not able to absorb moisture from soils which have an apparent specific gravity of about 1.6 for clays to about 1.8 for sands.

The concentration of the soil solution in most soils is so low that it has but little effect on absorption, but in strongly saline soils, absorption may be retarded to a considerable extent. The addition of fertilizers in amounts ordinarily used does not affect absorption of water from the soil.

Absorption may also be affected by the depth of rooting and is generally less near the limit of root extension where the soil is not thoroughly occupied by the roots. As an example, unirrigated walnut trees at Davis, California on a deep loam absorbed the readily available soil moisture in the top six by July 15 ; in the six to nine foot depth by August 19 ; and in the nine to twelve foot depth by september 14. In another case, almond trees on a deep loam absorbed all of the readily available moisture from the winter rains and three irrigations in the top nine feet by September 1, but only reduced the soil moisture by 2.5 percent in the nine to twelve foot depth during the entire growing season.

A discussion of the effects of leaf area, soil temperature, and soil aeration on absorption of water from the soil may be found in any standard textbook on plant physiology and will not be discussed here.

Annual Crops.

The absorption of moisture by the roots of annual crops is affected by the same conditions as those for perennial crops, but it cannot be measured accurately with young plants, the roots of which have not permeated the soil. Sometimes root growth in young plants is not rapid enough to supply the water needs of the plant, and wilting may occur even though soil samples a short distance away may show readily available moisture.

Methods of Measuring Absorption from the Soil.

Many methods for measuring absorption from the soil have been used, but the one we have found best suited to our needs is the systematic sampling of the soil at regular intervals. Sampling may be done either with an auger or a soil tube. Many other devices have been proposed to measure soil moisture but none have been entirely satisfactory through the entire range of soil moisture between the field capacity and the permanent wilting percentage, and some have additional disadvantages. In greenhouse experiments with plants in containers, weighing the entire system is satisfactory. Determination of the water absorbed by the roots, through the losses found in the moisture content of the soil, presents certain advantages. The calculations are on the oven dry weight of a given soil which is a constant. The fluctuations in moisture content of the soil, resulting from successive waterings and absorption by roots fall between the two constants previously described. In other words, the readings for each series have the same starting point and the same end point. The water holding properties of the soil being constant, variation in the amount and rate of absorption can be attributed to one or more of the factors affecting absorption which are given in previous paragraphs.

ELECTRICAL RESISTANCE METHODS AS FINALLY PERFECTED FOR MAKING CONTINUOUS MEASUREMENT OF SOIL MOISTURE CONTENT UNDER FIELD CONDITIONS (1)

by George John Bouyoucos

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In ecology as well as in the whole field of agriculture and in other fields, there has always been a great need for a satisfactory method of measuring the moisture content of soils.

At the Michigan Agricultural Experiment Station, in an effort to find a suitable method to measure and study soil moisture in the field, the plaster of paris and the nylon electrical resistance methods of soil moisture determination have been devised.

Plaster of Paris Electrical Resistance Method. The plaster of paris method of moisture determination makes it possible to make a continuous measurement of soil moisture in the field electrically. It utilizes two units, a small porous absorbent block and a modified Wheatstone bridge or impedance meter. Cast in the block are two parallel electrodes with leads. Such a block, when buried in the soil, becomes, to all intents and purposes, a part of the soil. It absorbs moisture from the soil and gives it up to the soil very readily, so that its moisture content tends to stay in constant equilibrium with the moisture content of the soil. The electrical resistance of the block varies with its moisture content, and that in turn varies with the moisture content of the soil in which it is buried. Therefore, by calibrating the blocks the moisture content of the soil may be determined by measuring the electrical resistance of the block. Figure 1 shows the relationship between the electrical resistance of the block and the total moisture content of a typical soil. The key to the success of this block lies in the fact that the electrodes are covered or imbedded in plaster of paris.

A very important aspect of this electrical resistance method is that it actually measures moisture tension. Its easily effective range is from field capacity or about 600 ohms resistance to approximately 2,000,000 ohms. In the soil moisture free energy concept this represents the equivalent range of tension from 0.3 atmospheres of pressure to 20 atmospheres of pressure. On the basis of this free energy concept, soils at any given electrical resistance level, hold their water with the same force or tension.

(1) Contribution from the Soil Science Department of the Michigan Agricultural Experiment Station, East Lansing, Mich. Authorized for publication by the Director as Journal Article 1595.

There are two soil moisture constants illustrated in Figure 1. These constants are important in discussing soil moisture in relation to plants. The first, is the permanent wilting point (W. P.). This value falls on the moisture curve at about 100,000 ohms resistance. As is well known, the permanent wilting point represents the moisture content in which plants wilt and die. The tension with which the water is held at this point is equivalent to about 16 atmospheres of pressure.

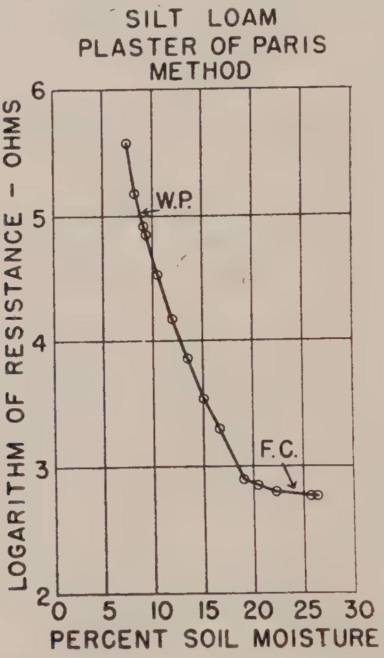

Figure 1. — The relationship between electrical resistance of the absorption block and the total moisture content of a silt loam soil.

The second soil moisture constant is field capacity (F. C.). This value falls on the moisture curve at about 600 ohms resistance. Field capacity is the maximum amount of water a well drained soil can retain under normal field conditions, and is roughly the moisture a field soil contains about 3 days after it has been soaked by rain or irrigation, the excess water having been drained away and evaporation having been prevented. At field capacity the water is held by the soil with a tension equivalent to 0.3 atmospheres of pressure.

The original plaster of paris blocks, have recently undergone some very important changes and improvements. In the first place, the latest blocks have a different type of electrode, which is thick, short and spaced close together. Undoubtedly, the most important improvement that has been made on the original plaster of paris blocks is to make them more durable.

By impregnating the plaster of paris blocks with alcohol-soluble nylon resin their life is prolonged more than 5 times.

As an example of the actual performance of the plaster of paris blocks under field conditions, Figure 2 is presented. The experiment was conducted under normal agricultural practices. Electrical resistance readings were taken daily by the special soil moisture bridge which will be discussed later. The chart shows the rapid response the absorption blocks made to the changes in the soil moisture content.

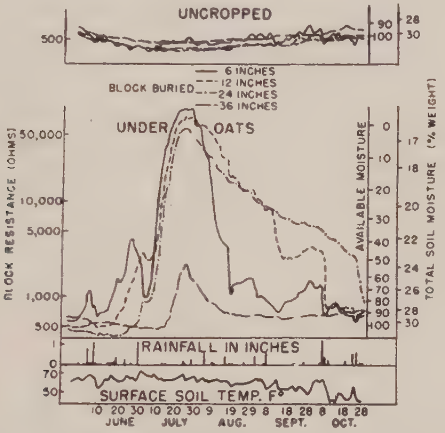

Figure 2. — Showing daily electrical resistance of a clay loam at different depths, uncropped and under oats, and the relative amount of available water at any given resistance reading, as well as the total moisture content at 6 inches depth.

The Nylon Electrical Resistance Method. The development of the nylon unit is the result of an extensive and intensive search made to find a unit superior in performance and durability to that of the plaster of paris block. Of all the materials examined and studied, the nylon fabric proved the most satisfactory.

Out of the nylon fabric, therefore, was constructed a soil moisture measuring unit. It consists of two pieces of stainless steel screen acting as electrodes, to which are silver-soldered wire leads. These electrodes are separated and wrapped by three single pieces of nylon fabric. The whole assemblage is then placed in a perforated stainless steel case and subjected to a uniform and controlled high pressure. While the center of the unit is being held under constant high pressure the edges of the unit are mechanically united to hold the enclosed assemblage permanently and in intimate contact.

Like the plaster of paris block, the nylon unit absorbs moisture from the soil and gives it up to the soil very readily. When the nylon unit is buried in the soil, its moisture content tends to be in equilibrium with that of the soil. The electrical resistance of the unit varies with its moisture content and, by calibration, becomes an index of moisture in the soil.

An interesting and valuable feature of the nylon units is their outer metal case, which acts as a shield and almost entirely eliminates electrical lines of force or « stray currents ».

The nylon units also have recently undergone some very important changes and improvements. In order to give the units a good contact surface at the grid, the nylon units, in their original form, are now encased in plaster of paris. The plaster of paris casing not only provides the nylon units with a better contact surface, but also provides a buffer action which minimizes the effects of salts.

Perhaps the most outstanding qualities of the nylon device are its marked sensitivity at high moisture contents, and the ability to measure moisture near the saturation range.

As a sample of the performance of the nylon method under actual field conditions, Figure 3 is presented.

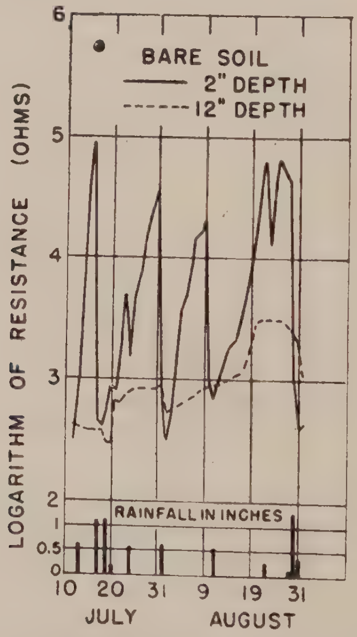

Figure 3. — Sample of performance of the nylon unit under field conditions.

Comparative Merits of the Plaster of Paris and Nylon Methods. The two methods will supplement each other, one covering the higher levels of water content, and the other the lower levels. However, the nylon method does give great promise of replacing the plaster of paris method.

Effect of Salts in the Soil on the Electrical Resistance Methods. Appreciable amounts of soluble salts in the soil would be expected to affect the accuracy of both the plaster of paris and nylon methods since both function electrically. However, there are several factors which tend to modify or minimize the adverse effects of salts. They may be listed as follows :

(1) Plaster of paris ($CaSO_4$) is soluble in water to the extent of 2200-2500 parts per million. This solubility factor provides a buffer action which tends to modify or minimize the effect of salts on the electrical resistance readings.

(2) Experiments have revealed that the effects of salts on the accuracy of the electrical resistance methods are most prominent near the saturation point. Below field capacity the effects of salts become unimportant.

(3) The process of leaching, capillary rise of salts, addition of fertilizers, removal of nutrients by plants, continual renewal of soluble salts, etc., tend to compensate each other and thereby minimize the effect of salts on the electrical resistance readings in the humid regions.

(4) If necessary, each soil can be calibrated and such calibration takes into account the physical-chemical characteristics and salt content of the soil.

Electrical Measuring Instruments. Two electrical maesuring instruments are used in connection with the plaster of paris and nylon units :,(1) a soil moisture bridge and (2) a moisture meter. The bridge is a modification of the Wheatstone bridge. The moisture meter is an alternating-current impedance meter based on the circuit of the special soil moisture bridge.

These electrical resistance methods have proven successful tools for the agronomists, civil engineers, highway engineers, hydrologists and others and are now widely used.

SOME RELATIONSHIPS BETWEEN THE SOIL WATER TABLE AND THE COMPARATIVE ECOLOGY OF *RANUNCULUS BULBOSUS*, *R. REPENS* AND *R. ACRIS*

by J. L. Harper

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The three closely related species of Ranunculus, *R. bulbosus, R. acris* and *R. repens* of the same division Chrysanthes are frequently found associated together in pasture and meadows. Attempts have been made to discover whether these species differ in habitat requirements, and so avoid inter-specific competition.

A characteristic feature of British grassland is the frequency of occurrence of ridge and furrow which give a pattern of drainage conditions over the fields. Analysis of the distribution of the three buttercup species where they occur in the same field, shows *R. bulbosus* on the tops of the ridges, *R. acris* on the sides and *R. repens* in the furrows. This arrangement may be modified when drainage is good ; *R. repens* may then be absent and the other two species occupy the furrow and ridge side, or when drainage is poor, *R. bulbosus* may be absent, and *R. acris* then colonises the tops and sides of the ridges.

Experiments were made in which 25 cm. pots were filled with soil and maintained at constant water table (a) at the surface, (b) at 12 cm. and a control series (c) was allowed to drain freely. Mature specimens of the three species were taken from established grassland and planted in the water table pots ; after 1 year's growth these transplants showed no differences in morphology or reproductive capacity associated with differences in water table. However, seeds of the three spp. sown in the late summer showed remarkable differences in early establishment. *R. bulbosus* gave best germination in fully drained pots, *R. acris* in the fully drained and half waterlogged pots, and *R. repens* gave the best establishment of all species in the fully waterlogged conditions, although the best germination was with a water table at 12 cm. When seeds of the species were sown mixed, then difference in water table effected a remarkable control over the balance of species developing.

It is concluded that a major factor influencing the environment suited to these three species is the soil moisture condition at the time of sowing, and that this rather than any effect on mature plants is largely responsible for the limitation of each species to a particular microhabitat in permanent grassland.

L'EVAPOTRANSPIRATION REELLE AU CHAMP : MESURE ET INTERPRETATION

par Marc **Hallaire**

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Les formules climatiques, telles que celles de Thornthwaite, appliquées au calcul de l'évapotranspiration E_c, mettent en évidence au cours de l'année la phase de réhumectation du sol, où les pluies excèdent E_c et où il y a drainage, et celle de dessèchement où, les pluies étant inférieures à E_c, la plante épuise les réserves du sol et augmente ainsi progressivement le déficit en eau. Mais ces formules ne traduisent qu'une tendance et leur limite de validité, en cas de sécheresse notamment, nous semble avoir été

définie de façon trop arbitraire et trop générale, au moins à l'échelle agronomique.

Or, les deux facteurs de l'alimentation en eau, qu'il suffirait de pouvoir mesurer régulièrement pour résoudre maints problèmes, sont le déficit total en eau et ce qu'on doit appeler la sécheresse du sol :

a) *Le déficit D,* quantité d'eau nécessaire pour ramener à toute profondeur Z l'humidité H à la capacité au champ H_0, peut se mesurer directement sur un profil en H, en évaluant, pour chaque couche, par la méthode classique de prélèvements d'échantillons et dessèchement à l'étuve, l'écart entre H et H_0.

Son intérêt, indépendamment des problèmes d'irrigation, est d'indiquer par différence, et compte tenu de la pluie, la valeur de l'évapotranspiration réelle E. Par contre, il ne définit pas le degré de sécheresse puisque ce dernier variera énormément d'un sol à l'autre pour un même déficit.

b) *Le degré de sécheresse.* Il est représenté à chaque profondeur par le potentiel capillaire ψ, ou ce qui revient au même par $\log\psi$ qui n'est autre que le pF. On peut l'évaluer directement au champ par la résistance électrique de certains éléments hygroscopiques, en nylon par exemple.

Or, nous avons montré (1) qu'au cours de la période de dessèchement, phase active de la végétation sous nos climats, une seule mesure de ψ, soit une mesure de résistance électrique R_1, réalisée à un niveau donné Z_1, suffit à définir ψ à toute profondeur Z.

Dès lors, on peut se référer à cette unique mesure R_1 pour apprécier globalement les degrés de sécheresse de différents sols, les comparer et préciser les conditions d'une alimentation en eau déficiente.

Mais, en outre, cette mesure R_1 qui suffit à définir tout le profil ψ (Z), définit par là même, pour un sol donné, l'ensemble du profil en H et, par conséquent, le déficit D. Ainsi voit-on la possibilité d'établir pour chaque sol, et par étalonnage direct au champ, une relation entre le déficit total D et le degré de sécheresse R_1, puis de déduire la valeur du déficit D d'une simple mesure électrique.

Nous voudrions montrer maintenant que si les évaluations régulières de D à partir de R_1 permettent de mesurer, au cours du dessèchement, l'évapotranspiration réelle E, la méthode proposée conduit aussi à mettre en évidence le rôle de la plante et du sol dans l'alimentation en eau du végétal.

En représentant graphiquement la variation dans le temps de E et de E_c relatifs à deux sols A et B que nous avons expérimentés, nous avons tout d'abord constaté un excellent accord entre ces deux valeurs tant que le sol était assez humide. Cela souligne l'intérêt des formules climatiques telle que celle de THORNTHWAITE que nous avons utilisée. Par contre nous avons observé qu'au-delà d'un certain moment, le déficit et par conséquent aussi la sécheresse pouvaient plafonner à certaines valeurs, l'évapotranspiration E étant dès lors limitée aux précipitations. C'est ici que notre méthode est susceptible d'apporter d'utiles renseignements.

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(1) M. HALLAIRE : *Thèse Ing.-Doct.*, Paris, 1953, n° 404, et *Ann. I.N.R.A.*, sér. A., 1953, n° 2, p. 143-244.

Elle permettra tout d'abord de préciser le *rôle du sol* sur les possibilités d'alimentation en eau offertes aux cultures. Il faut en effet remarquer que le végétal n'est sensible qu'au facteur sécheresse et c'est ainsi la valeur de R_1 et non de D qui fixe le moment où, la plante cessant d'épuiser les réserves en eau, E devient inférieur à E_c. Or le simple examen des courbes d'étalonnage (R_1, D) montre que pour une même valeur de R_1 (log R_{30} cm = 5,3 par exemple soit pF à 30 cm $\sharp$ 4,2) D peut être très différent d'un sol à l'autre (D = 120-140 mm pour le sol A et 60-70 pour B). Dans ces conditions, le déficit maximum D_M. au-delà duquel la formule climatique cesse d'être valable et dont l'intérêt est de représenter le stock d'eau disponible, est essentiellement fonction du sol. D_M sera normalement plus faible pour le sol B, où nous avons pu l'égaler à 120 mm environ (sous culture de blé), que pour le sol A où il dépasse couramment 200-250 mm.

Mais, par ailleurs, pour mettre en évidence le *rôle de la plante* et comparer le pouvoir desséchant des différentes cultures, il suffira de se référer à la sécheresse maximum (R_1) M qui, nous l'avons constaté, peut varier assez considérablement en fonction du couvert végétal.

Cette méthode pourrait également servir à préciser *l'influence* sur l'économie de l'eau de *certaines pratiques* telles que le sous-solage, la densité de semis ou la fertilisation en profondeur.

LE CALCUL DU BILAN D'EAU DES CULTURES ET LES FAITS PHYSIOLOGIQUES

par L. Turc

Laboratoires des Sols, C. N. R. A. Versailles

Nous avons montré que la hauteur annuelle moyenne de l'évaporation, E, sur un bassin de cours d'eau peut être évaluée en fonction de la hauteur annuelle moyenne des précipitations, P, et de la température moyenne de l'air, t, par la formule :

$$(\text{I}) \quad E = \frac{P}{\sqrt{0{,}9 + \dfrac{P^2}{L^2}}} \quad \text{avec } L = 300 + 25\,t + 0{,}05\,t^3 \qquad \begin{array}{l} t \text{ en } {}^\circ\text{C} \\ P \text{ en mm} \end{array}$$

Pour 254 cours d'eau situés en Europe, en Afrique, en Amérique, à Java, l'écart entre E ainsi calculé et la valeur observée est inférieur à 40 mm dans 53 % des cas. De plus, cette formule permet d'évaluer l'évaporation annuelle des cases lysimétriques portant des cultures avec une approximation presque du même ordre que celle obtenue pour les bassins de cours d'eau.

Pour obtenir une approximation plus poussée et suivre les variations au cours de l'année de l'évaporation et, selon les cas, de l'assèchement du sol ou du lessivage du sol, nous avons mis au point une méthode de calcul décade par décade.

Pour les cases dont le sol est nu, l'évaporation par 10 jours est donnée par la formule :

$$(II) \qquad E = \frac{P + a}{\sqrt{1 + \left(\frac{P + a}{l}\right)^2}} \qquad \text{avec} \quad l = \frac{(t+2)\sqrt{i}}{16}$$

i est la radiation globale, les calculs peuvent être faits avec les seules températures si i n'est pas connu ; « l » représente ici le facteur héliothermique comme L le représentait dans (I). E et P sont des hauteurs d'eau exprimées en mm relatives à une période de 10 jours, ainsi que l et a.

« a » est la hauteur d'eau susceptible d'être évaporée en 10 jours en sus des précipitations, donc aux dépens des réserves du sol ; pour un court intervalle de temps, en effet, on ne peut établir le bilan sans introduire les variations des réserves du sol ; la valeur de « a » utilisée pour chaque décade se déduit très simplement des résultats des calculs pour la décade précédente.

Ce calcul a été généralisé pour le sol portant une végétation, la formule devient :

$$(III) \qquad E = \frac{P + a + V}{\sqrt{1 + \left(\frac{P + a}{l} + \frac{V}{2l}\right)^2}}$$

« V » qui est fonction de la masse de la récolte représente l'effet de la végétation ; pour V = o on retrouve la formule (II).

Pour des installations de cases lysimétriques situées en France, en Angleterre, en Hollande, en Ecosse, en Afrique du Nord, aux Indes nous calculons en sol cultivé comme en sol nu les drainages annuels (sommes de 36 drainages décadaires) avec des écarts inférieurs à 40 mm en valeur absolue et à 20 p. 100 en valeur relative plus de deux années sur trois; les périodes de sécheresse pendant lesquelles le drainage est interrompu sont retrouvées par le calcul généralement à un mois près.

Enfin les quantités d'eau qu'évaporerait une végétation luxuriante et ne manquant jamais d'eau sont évaluées en faisant V = constante = 70 (ce qui correspond à une formation de matière sèche récoltable égale à 8q/décade/ha) ; les résultats ainsi obtenus fournissent une évaluation satisfaisante des besoins en eau d'irrigation.

En conclusion, les quantités d'eau évaporées par le sol et sa végétation pendant l'année, le mois, la décade, peuvent être évaluées par des calculs simples en fonction essentiellement de la hauteur des précipitations et de la température ; il faut insister sur le fait que la formule (III) donne généralement des résultats presque aussi satisfaisants si, au lieu de la masse exacte de matière végétale récoltée, on n'utilise qu'une évaluation grossière de cette masse, et si l'on calcule « V » sans tenir compte de la nature de la végétation.

La formule (III) est fondée sur l'idée que la végétation joue le rôle d'une mèche mettant une partie de l'eau du sol à la disposition du pouvoir évaporant de l'air ; celui-ci peut donc produire une évaporation plus intense que celle qui se produit lorsque le sol est nu et surtout le sol peut être asséché plus profondément. Si la végétation est très maigre, l'évaporation diffère peu de celle du sol nu, si la végétation est abondante et le sol bien pourvu en eau, l'évaporation est proche de celle d'une nappe d'eau libre ; dans les autres cas l'évaporation a une valeur intermédiaire qui est voisine du maximum compatible avec l'approvisionnement en eau évaporable, dans la mesure où la végétation est suffisamment développée pour assurer largement l'effet de mèche ; si cette condition est remplie, un nouvel accroissement de la densité de la végétation n'accroît guère l'évaporation. Pour ces raisons, la masse de la récolte intervient seulement sous un radical dans le calcul de V et, par ailleurs, on introduit dans ce calcul un terme qui croît depuis la levée jusqu'à la date de la récolte, afin de tenir compte de la croissance du système radiculaire.

Le problème du bilan d'eau des sols a donc pu être traité moins comme un problème complexe de physiologie que comme un problème physique relativement simple. Les aspects physiologiques ne passent au premier plan que dans une étude plus fine du phénomène, et lorsqu'on envisage non pas le bilan d'eau pour lui-même, mais l'adaptation de tel végétal au climat défini par ce bilan d'eau. Des cultures différentes n'ont pas la même résistance aux excès d'humidité de l'atmosphère ou du sol, ni à la sécheresse, alors que les quantités d'eau qui leur seraient nécessaires pour maintenir une activité biologique maximale assurant des récoltes très élevées sont approximativement les mêmes (1) ; c'est pourquoi nous pouvons évaluer

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(1) L'olivier peut subsister en climat très sec, il « permet d'exploiter un milieu très pauvre ; c'est une machine qui ne fonctionne que quand les conditions le

les besoins en eau d'irrigation sans tenir compte de la nature de la culture, mais seulement du climat et de la période de végétation.

⁂

DO PLANTS ABSORB WATER AGAINST A GRADIENT ?

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In order to remove it from the field of semantics, active water absorption is defined as the movement of water into the plant from a region of lower activity (or free energy) of the water molecules to a region of higher activity (or free energy). Any other meaning that might be assigned to the term would make it superfluous and unsound.

Since the root is the water absorbing organ, par excellence, it is the logical place to look for such negative gradients. It is, of course, now thoroughly established that the absorption of ions by roots is active — *i. e.* against a gradient. The activity of the ions in the absorbing root cells is therefore greater than their activity in the surrounding medium. Since any solutes decrease the activity of the solvent, it follows that the activity of the water in the roots cannot be greater than that of the water in the soil.

But is it possible that other cells in the plant (e. g. the endodermal cells) do not actively absorb solutes but do actively absorb water ? For a conclusive answer of general application, a theoretical approach to this problem is necessary.

The existence of active ion absorption is often taken as evidence that active water absorption may also occur. As pointed out above, the two are mutually exclusive, since the existence of negative gradients for ions prevents the existence of negative gradients for water, and vice versa. But there is another important difference between the absorption of ions and of water. Active ion absorption is via a « one-way valve ». The ions are pulled through the plasma membranes into the vacuole, but cannot leak out of the vacuole again because the membrane is impermeable to them. Once in the vacuole, no more energy is needed to keep them there ; and one can maintain such cells in distilled water almost indefinitely without loss of ions, even at temperatures near freezing, at which respiratory release of

permettent et qui peut s'arrêter sans se détraquer » ; mais il bénéficie largement d'apports d'eau supplémentaire ; en Tunisie la production d'huile passe de 300 kg. par ha. et par an pour une pluviosité annuelle de 200 mm., à 1.200 kg. si les précipitations et l'irrigation apportent 800 mm. d'eau. (A. VERNET, *Oléagineux*, juillet 1953, p. 434.)

energy is negligible. In the case of water molecules, the « one-way valve » does not operate since the plasma membranes are freely permeable to water in both directions.

Consequently, if the activity of the water molecules inside the vacuole were raised above that of the cell environment, water would begin to diffuse out, and enough energy would have to be *continuously* expended by the cell to continue pulling water molecules into the vacuole as rapidly as they diffused out.

It was previously shown (Levitt, 1947) that this energy can be calculated from the formula :

$W = 16^6 p^2 AP$ ergs/hr./g. tissue. Where W = work done, p = negative pressure against which the water is moved, A = specific surface across which the gradient occurs, P = permeability of the cell to water.

In one paper, the protoplasm was stated to maintain an osmotic potential 5 atms. higher than that of the vacuole of the same cell. Calculations from the above equation showed that the cell would very soon respire itself to death by maintaining such a gradient. It was not surprising, therefore, to find that the experimental methods used to determine the values for protoplasm and vacuole were at fault.

Nevertheless, a recent paper (Bonner et al.) has produced the same assertion — that water can be absorbed against a negative osmotic potential of 7 atms. ! It is obvious that the cells would respire themselves to death even more rapidly than in the above case. Furthermore, when they doubled the supposedly negative osmotic potential difference (from 2.4 to 4.8 atms.) the IAA-induced « active » water uptake was reduced to half, and the IAA-induced increase in respiration dropped from 50 % to 25 %. But it will be noticed from the above equation that the work done in moving water against a gradient is proportional to the square of the negative osmotic potential difference. Consequently, doubling the negative gradient should require 4X as much energy for the same amount of water actively absorbed, or 2X as much for half the amount. Thus, if they were really dealing with active water absorption, the « active » respiration would be expected to increase by 100 % instead of dropping by 50 %.

As in the previous case, the thermodynamic proof of error is fully confirmed by recent evidence that their experimental results were at fault. Burström has shown that their osmotic values are far too low, and the supposedly hypertonic solutions were actually hypotonic. Plasmolytic determinations by the author have confirmed Burström's results. Burström also showed that the plasmolyte they used penetrates the cells, thus vitiating their results.

Due to its dependence on the active absorption of solutes, the passive absorption of water would, of course, be indirectly dependent on aerobic respiration. The consequent dependence of water absorption on oxygen has also been offered as evidence that an active process is involved (Rosene).

Here again, the results cannot possibly be explained by active water absorption (LEVITT 1952).

The conclusions we are faced with are as follows : a) Negative water gradients do not exist between the water absorbing roots of plants and their environment. b) No plant cells produce enough energy to maintain such gradients. c) Experimental evidence claiming to prove active water absorption is due to error in determinations or in interpretations of results. With this concept out of the way, the search for the true explanations of such phenomena as the mechanism of auxin action, etc., is more likely to be fruitful.

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ACTIVE WATER UPTAKE BY PLANT CELLS

by A. S. CRAFTS

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In any consideration of the water economy of plants, attention should be given to the forces that influence water uptake, water retention and water loss. Classically, water uptake has been explained on purely physical grounds ; osmosis has been given as the predominating process involved.

For over 10 years, evidence has been accumulating that casts some doubt on the adequacy of osmosis as a process to explain rapid water uptake during growth, to account for the frequent discrepancy between the osmotic pressure of cells determined plasmolytically and cryoscopically, to explain the role of auxin in water absorption, and to rationalize the role of numerous respiratory inhibitors in the regulation of water balance. In 1949, CRAFTS, CURRIER and STOCKING reviewed the subject, pointing out the inadequacy of evidence to back the various claims for active water uptake.

Since that review numerous papers have described experiments showing that addition of auxin will bring about absorption of water. This is attended by growth, and the cause : effect relation between the two has not been clarified. Furthermore, increased water uptake from added auxin does not prove that normal water content necessarily results from the action of endogenous auxin.

Several have attempted explanations of active water uptake on the basis of permeability changes. To one who has worked with the plasmolytic technique, any such dynamic view seems untenable in terms of the rapid equilibria normally attained. Any process taking place over several days and resulting in a permanent or irreversible increase in cell and tissue dimensions would seem to demand at least steady state conditions, and in the case of tuber tissues, mechanisms capable of maintaining static conditions of turgor. Here one should make a clear distinction between the role of active water uptake, and the role of permeability in explaining turgor in excess of that attributable to osmotically active substances in the vacuoles. Such a distinction should be made between water uptake and the over-all process of growth. And, finally, one should understand that an accumulation of water presupposes a disaccumulation of salts and vice versa.

One aspect of the problem that deserves more attention is the hydration state of the cell cytoplasm. Currier has stressed this in this publications and is working on it in his present studies. Often the cytoplasm occupies a larger portion of the total cell volume than is ordinarily assumed, and the cytoplasm : vacuole volume relation may change rapidly. This point needs checking in all studies on plant cell water relations.

From the standpoint of general plant physiology including growth, active water uptake is an attractive mechanism for explaining many phenomena. And for certain agricultural fields such as vegetable and flower production, where turgor is an important factor in quality, mechanisms for attaining and maintaining high turgor are of great importance. In contrast, in those aspects where seed and fiber production are concerned and in those fields of plant ecology that deal with water absorption and utilization from soils of relatively low water content, and particularly in dealing with drought resistance and drought survival, it seems that mechanisms of water retention are of paramount importance. Forces involved in water retention near or at the permanent wilting percentage are of a different order than those postulated for active uptake. And mechanisms responsible for survival of tubers, seeds, and other storage organs involve prevention of loss against great force rather than uptake or maintenance in a fully hydrated state. In dealing with problems of plant water relations these facts should be kept in mind.

THE WATER ABSORPTIVE CAPACITY OF ROOT HAIRS

by Hilda F. Rosene

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Until 1940, no successful attempt appears to have been made to prove by direct means that the root-hair actually absorbs. At that time the writer devised a micropotometric method which has supplied quantitative data, not only on the rates of water entry into different regions of a single hair, different hairs on a single root, and hairs of different species, but it also provided a means to study the effects of drugs when these were applied to only a portion of a single hair. Since the micropotometer simulates a soil pore space filled with water, the method is useful to study problems connected with water and ion intake.

In the first study, it was shown that the speed of water entry into radish root-hairs was highest at the distal end of the hairs. Other studies on radish and on wheat seedlings showed that the water absorptive capacity slows down in older hairs as they increase in length. The fact that water entry shows down with ageing of the hair raises the interesting question of whether or not older hairs collapse and die when they no longer function in absorption. It would seem that the greater capacity of the hair tip and the greater capacity of the younger hairs to absorb water as shown by higher rates per unit surface area, would play important roles in the water economy of some plants in relation to daily or seasonal new root and root-hair development, especially in field grasses and small grain crops which have extensive root systems with numerous hairs.

It has been assumed by some workers that the hairless epidermis is as readily permeable to water as the root-hair surface. Work on the root-hairs of onion seedlings and of bulb roots show that the rates of water entry into the hairs are indeed comparable to the rates of water entry through the epidermis or hairless onion roots. Comparatively few hairs absorb water at speeds as high as those found at the highest levels of intake rates along the hairless root axis. But the average speed of entry into hairs at the distal regions of the haired root is higher than the average rates of intake in the epidermal cells in the same region of hairless roots. When the haired roots were excised, absorption by the hairs was equal to, higher, or lower, than intake before excision. Studies on osmotic pressure show that, in general, the range of critical concentrations of sucrose and KNO_3 which will balance the internal factors that operate to determine the speed of water entry into onion root-hairs is similar to that observed earlier for hairless onion roots.

At temperatures above 25° C, single root-hairs of oats, wheat, and barley absorbed water intermittently, periods of water intake alternating with periods of water loss.

Oxygen seems to be necessary, not only for development of, but also for water intake by the hairs. In nitrogen the absorptive capacity of radish root-hairs was reduced and with prolonged exposure, water entry ceased. Exposure of a portion of a root-hair to azide resulted in inhibition of water entry ; these effects were largely localized in the hair. The experiments with oxygen lack and azide show that the entry of water in root-hairs in a moist atmosphere is dependent on oxidative metabolism.

In a study on 10 species, the highest mean rates of water entry were round in the root-hairs of field pea, tomato, and radish ; the lowest in wheat, oats, and barley. Corn hairs showed intermediate rates as did the hairs of Chinese cabbage, Georgis collard, and mustard. Data on the rates of water intake by rye root hairs were combined with published data from Dittmer on the total root-hair surface of the root system of a mature rye plant, and with data reported by Briggs and Shantz on transpiration. The calculated average absorptive capacity of the root-hair system for 24 hours amounted to 55 times the maximum transpiration rate. Hence, on a theoretical basis, maximum transpiration needs could be met if the average rate of intake of the entire root-hair system were reduced by 1/55 or if water entered only 1/55 of the total root-hair surface. If absorption at similar rates occurred through both the haired and hairless surface of the daily new root growth it would be theoretically possible to balance the loss by maximum transpiration, and at lower transpiration rates, absorption by the new root hairs would be sufficient. As Kramer and Coile stated, at least under some conditions root extension might supply all the water required by a plant.

When available moisture is irregularly distributed in a soil penetrated by an extensive root system with many hairs, the water needs of a plant might be supplied by normal activity of a few of the total number of hairs or by maximal activity of only a portion of each hair out of the total number, particularly if only the hair tip contacts water. Perhaps hair absorption is very important under conditions of atmospheric drought and high transpiration as well as at other times.

CRITIQUE EXPERIMENTALE DE DEUX METHODES EMPLOYEES EN VUE D'ETABLIR LE DEFICIT DE SATURATION HYDRIQUE (DSH) DES FEUILLES

par H. R. Oppenheimer
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D'après Stocker (1929), le DSH est déterminé en chambre humide par immersion dans l'eau des pétioles de feuilles intactes, tandis que Weatherley (1949) en tire des rondelles qui se saturent en flottant sur l'eau. W. définit

comme « turgescence relative » (TR) le rapport de l'eau au champ au taux de saturation, exprimé en pourcentage de ce dernier. En calculant (100-TR), on obtient les DSH de Stocker rapportés aussi au taux hydrique à saturation.

Examinant des feuilles de pomélo (*C. paradisi*), de cotonnier (*Gossypium hirsutum* « Cocker 100 ») et une sélection locale d'*Helianthus annuus* à l'aide des deux méthodes, notre élève J. Nitzan, qui a exécuté les expériences, et l'auteur, qui a complété leur interprétation, sont arrivés aux conclusions suivantes :

1) Dans les conditions anisothermiques du transport au laboratoire de feuilles ou branches feuillées non pesées auparavant, on observe souvent des échanges de vapeur d'eau entre les parois du récipient (doublées de papier ou non) et les premières. C'est pourquoi des enveloppes imperméables de nylon, à poids prédéterminé, sont préférables à des récipients de verre, fer-blanc, etc... non tarés.

2) En accord avec les conclusions de Härtel (1936) et Welten (1933), le rapport du taux hydrique au champ au taux « maximum » apparaît problématique, car le dernier varie souvent suivant la méthode. En général, il est de beaucoup supérieur pour les rondelles que pour les feuilles puisant l'eau par les pétioles, et il en résulte des valeurs de DSH plus hautes par la méthode de Weatherley. Le contact immédiat avec le liquide en semble être responsable.

3) En opposition aux résultats de Weatherley, Nitzan a établi que des rondelles flottant en position normale, c.-à-d. avec leur face inférieure plongée, s'infiltrent souvent d'eau par leurs espaces intercellullaires, et surtout à l'obscurité. Comme ce phénomène, très prononcé chez le pomélo, n'est observé chez cette espèce ni à la lumière ni en position inverse, il résulte probablement de la respiration avec stomates bloqués par l'eau. La forte absorption par l'eau du CO^2 exhalé produit un vide aux interstices du mésophylle qui aspire de l'eau à la périphérie des rondelles. Le même phénomène observé sur les feuilles amphistomatiques de *Gossypium* et *Helianthus*, serait associé avec un degré d'hydrophilie différent des cuticules aux faces opposées (tournesol) ou bien avec le caractère spongieux du tissu lacuneux à la face inférieure (coton). Chez ces espèces, l'orientation inverse des rondelles qui s'incurvent dans l'eau ne suffit pas à éviter l'infiltration. Il faut les mettre sur papier-filtre sursaturé d'eau, dans des boîtes de Pétri.

4) La facilité de l'accès de l'eau caractéristique de la méthode des rondelles explique leur vitesse de saturation supérieure à celle des feuilles intactes. En fonction du temps, on obtient — chez des objets sains et en conditions extérieures naturelles — de belles courbes de poids logarithmiques pour des rondelles et des feuilles. Chez le pomélo, la saturation des rondelles est pratiquement terminée après 3 à 5 heures, à des températures constantes entre 5 et 32° C, tandis que la saturation par le pétiole exige 24 heures, entre 12 et 32°, et plus à 5°. Les valeurs définitives dépendent

peu de la température qui accélère l'absorption de l'eau. Les résultats évoquent l'idée d'achever la saturation toujours à des températures constantes ajustées aux adaptations spécifiques des plantes étudiées. De cette façon on éviterait aussi tout échange de vapeur d'eau pendant la saturation. Chez *Gossypium* et *Helianthus,* la saturation des rondelles sur papier-filtre exige 10 à 30 heures, celle des feuilles 24 heures.

5) Des rondelles tirées de différentes régions de la même feuille ne contiennent pas le même pourcentage l'eau. Evitant les nervures, on enlève essentiellement du trophenchyme moins hydraté que le tissu vasculaire au même degré de saturation. Le taux hydrique diminue de la base au sommet (pomélo) et, d'ordinaire, le DSH s'élève au fur et à mesure que le tissu enlevé est plus éloigné du système vasculaire. Ces différences nuisent à la comparaison de résultats obtenus par les deux méthodes et démontrent la nécessité de standardiser les procédés d'enlèvement des échantillons.

6) Un inconvénient de la méthode de STOCKER est la perte de substance organique pendant la saturation qui, chez les objets étudiés, atteint 1 à 2,5 % de la substance fraîche (WEATHERLEY les élimine par le calcul). En outre, des feuilles d'insertion et structures analogues rendent souvent des résultats très différents (cf. ARVIDSSON, 1951). — La méthode des rondelles ne s'applique pas à des feuilles petites et poilues ; les courbures ou l'infiltration la rendent souvent inapplicable. Or, employées avec discernement, l'une et l'autre méthodes rendront de bons services. Cependant, tout objet demande une étude préliminaire ; l'application des mêmes méthodes à des objets quelconques a contribué au discrédit de l'écologie expérimentale comme science exacte et doit être éliminée.

OSMOTIC PRESSURE AND SALT PERMEABILITY OF THE CELLS OF THE MOULD, WHICH GROWS ON THE GLASS SURFACE

by Torao OHTSUKI

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Lenses and prisms of optical instruments are often grown by a mould, which was named by the author « *Aspergillus glaucus* var. *tonophilus* ». The mould does not grow on media unless salts or sugars are added in a large amount, which seems to indicate the fungal cells having an enormous high osmotic pressure. Osmotic pressure of the cell sap estimated is always higher than that of the medium, for the more concentrated the medium is, the greater is the osmotic pressure of the cell sap. Differences between inside and outside of the cell amount to about 1-2°C on an average. NaCl was added to 3-22 % to Koji-medium. Richards' medium, to which KCl was added to 13-16 % was also used.

The osmotic value of the fungal cells, which grew in 24 % NaCl containing solution, showed depression — 21°C, that corresponds to the value of at least 300 atmospheric pressure. There is existing some parallelism between the ability to grow on glass surface and obligatory tonophily.

Experiments on salt permeability of the cell were tried to know the reason why the pressure is so highly risen through adding osmoactive substances. It was proved that NaCl and $(NH_4)_2SO_4$ are able to penetrate the plasma membran.

THE REGULATING INFLUENCE OF SUCTION TENSION AND TRANSPIRATION ON THE WATER AND SALT UPTAKE

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According to the formula of **Brieger** (1928) : Uptake $= g\,(S_{\text{vessels}} - S_{\text{medium}})$, the water uptake is determined by the difference between the suction tension in the vessels and the suction tension in the medium, and by the conductivity of the root tissue (g).

By increasing the transpiration the suction tension in the vessels is enhanced. Assuming the conductivity to be constant the increase in water uptake will be proportional to the increase in suction tension. Several authors have proved that this is not the case. **Brewig** (1937) found that by enhancing the transpiration the water conductivity of the root tissue increases too. These changes in conductivity especially take place in the more basal root regions. **Brouwer** (1954) showed the possibility to obtain similar changes in conductivity by applying an osmotic suction tension in the outer solution. This increase in conductivity was supposed to be due to a decreased turgidity of the root tissue. So transpiration influences the water uptake in two different ways. There is a direct influence on the suction tension in the vessels and there is a secondary effect by which the water conductivity is increased.

Further, experiments are reported upon the relation between water and salt uptake. In cases where a correlation between water uptake and salt uptake is found, it is possible to demonstrate the independence of these two processes by inhibiting one of them without influencing the other one. By means of respiration inhibitors the salt uptake can be reduced while at the same time the water uptake remains unaltered. It appears that in contrast with the salt uptake, water uptake is independent of respiration.

On the other hand it is possible to diminish the water uptake by applying an osmotic suction tension in the medium without any reduction of the salt uptake.

Experiments indicate that as a result of an increased transpiration the conductivity of the root tissue for salts increases in the same way and to the same degree as the conductivity for water does. The occurrence of a correlation between water and ion uptake proved to be dependent on the salt status of the plant material. Salt uptake wil be promoted by an increased transpiration if the salt absorbed can also be utilized. Otherwise utilisation will be limiting for the rate of salt uptake.

RÉSISTANCE A LA SÉCHERESSE

RAPPORT

DIE TROCKENRESISTENZ DER PFLANZEN
LA RESISTANCE DES VEGETAUX A LA SECHERESSE

von Otto Stocker
Darmstadt

I. BEGRIFFSBESTIMMUNGEN

a) Vitale und letale Trockenresistenz

Trockenheit kann Pflanzen unmittelbar töten (letale Effekte) oder nur ihre Lebenstätigkeit stören (vitale Effekte), woraus dann allerdings sekundär tötliche Wirkungen entstehen können. Da die Ausdrücke « Austrocknungs » — und « Dürreresistenz » sprachlich unbestimmt sind und widerspruchsvoll gebraucht werden (Levitt 1951) wollen wir von « *letaler* » und von « *vitaler* » *Trockenresistenz* sprechen, wobei « trocken » die verschiedensten Grade von leichter *An*trocknung bis zu völliger *Aus*trocknung bedeuten kann. Die vitalen Effekte haben eine grosse Breite und umfassen zahlreiche, zunächst nicht lebensgefährdende Vorgänge, wie etwa Anderungen von Atmung, Photosynthese, hydrolytischen Umsetzungen usw. Ihr Studium ist aus zwei Gründen sehr wichtig : *Physiologisch* liegen in ihrem Bereich die Wurzeln der Vorgänge, die zum unmittelbar-letalem Effekt führen. *Okologisch* kommt der vitalen Trockenresistenz eine mindestens ebenso grosse Bedeutung zu wie der letalen ; denn innerhalb der Trockengebiete entscheidet über die Verbreitung einer Art nicht der Trockentod in extremen Katastrophenjahren, dessen Verluste sich schnell wieder ausgleichen, sondern die vitale Konkurrenzfähigkeit in normalen Trockenjahren. Die Landwirtschaft meint mit « Dürreresistenz » überhaupt nur die einen ausreichenden Ertrag garantierende vitale Resistenz.

b) Plasmatische und konstitutionelle Resistenz

Wenn, wie bei vielen Thallophyten, die einzelnen Zellen in unmittelbarem Kontakt mit der Umwelt stehen, ist die Trockenresistenz eine rein *plasmatische* Angelegenheit. Bei manchen höher differenzierten Thallophyten, z. B. Pilzen, Flechten und Braunalgen, trifft das nicht mehr voll zu. Vollends bei den Kormophyten befindet sich das Plasma in einem gegenüber der Umwelt günstigeren und besser stabilisiertem Wasserzustand, welcher durch die kormophytische Konstitution ermöglicht wird. Wir unterscheiden deshalb zwischen « *plasmatischer* » und « *konstitutioneller* » Trockenresistenz, wobei der Ausdruck « konstitutionell » die Ganzheit der Strukturen und Reaktionen meint, welche auf den plasmatischen Wasserzustand Einfluss nehmen. Diese Begriffsbestimmung (STOCKER 1942) ist enger, aber sprachlich eindeutiger als die von BIEBL (1949), der mit konstitutionell die genotypische Komponente sowohl des Plasmas als auch der Gesamtorganisation meint.

c) Phänotypische und genotypische Resistenz

Jede Pflanze zeigt bei Antrocknung Dürreeffekte, die plasmatische und konstitutionelle Veränderungen im Rahmen der arteigenen Modifikationsfähigkeit mit sich bringen. Die dabei auftretende « Abhärtung » bedingt eine Resistenz, die ich im Anschluss an den Wortschatz der Genetiker als « *phänotypisch* » bezeichnet habe. Die phänotypische Reaktion ist begrenzt durch den Genotypus der Art, der hervortritt, wenn ich die Resistenz verschiedener Arten miteinander vergleiche : « *genotypische* » Resistenz. Diese Begriffe (SCHMIDT, DIWALD u. STOCKER 1940) entsprechen der von BIEBL (1949) gebrauchten « ökologische » und « konstitutionelle » Resistenz.

II. DIE PLASSMATISCHE TROCKENRESISTENZ

a) Die letale Plasmaresistenz

Die letale Trockenresistenz ist am auffallendsten bei den zahlreichen Thallophyten, die *völliges Austrocknen* ertragen. Bei den Kormophyten gibt es, abgesehen von den Farnen, nur wenige gesicherte Fälle, wie etwa den der südwestafrikanischen Scrophulariacee *Chamaegigas* (Heil). Wie aber schon Sporen und Samen zeigen, ist die prospektive Potenz zur totalen Austrocknungsresistenz des Plasmas in keiner Gruppe des Pflanzenreichs verloren gegangen und kann bei sehr schonendem und langsamen Vorgehen z. B. in Epidermiszellen des Rotkohls bis zur Exsikkatortrockenheit verwirklich werden (ILJIN). Normalerweise freilich ist die letale Plasmaresistenz bei den meisten Thallophyten und so gut wie allen Kormophyten auf mehr oder weniger hohe *An*trocknungsgrade begrenzt, woran teilweise eine unvollkommene Ablösbarkeit des Protoplasten von der Zellwand (ILJIN), teilweise aber auch eine weniger stabile Struktur des Plasmas Schuld sein dürfte. Eine scharfe Grenze zwischen dieser beschränkten *An*trocknungsund voller *Aus*trocknungsresistenz besteht nicht.

Eine *ökologisch* unmittelbar begrenzende Rolle spielt die letale Plasmaresistenz bei den *Thallophyten*, bei denen das Wasserpotential der Zellen fast ungehemmt den Schwankungen der Atmosphäre folgt. Bei Labermoosen hat sich eine genotypische Abstufung von Arten, die schon bei einem 24stündigem Absinken der relativen Feuchtigkeit auf 95 % zu Grunde gehen, bis zu solchen, die wochenlange Exsikkatoraustrocknung aushalten, ergeben, in Ubereinstimmung mit den ökologischen Verhältnissen der Standorte. (HÖFLER 1943, 1950) Ähnliches gilt für Meeresalgen (BIEBL 1952) und Flechten (LANGE). Nun kompliziert sich aber das Problem der letalen Resistenz nach zwei Richtungen : Einmal ist innerhalb genotypischer Grenzen durch Abhärtung oder Verweichlichung eine bisweilen ausserordentlich weite phänotypische *Modifikation* möglich, die z. B. bei Lebermoosen von 6 % bis zu 65 % r. F. gehen kann (HÖFLER 1950). Der Zustand des Plasmas, auf dem die letale Resistenz beruht, ist also bedingt durch vorhergehende *vitale* Dürreeffekte, sodass die letale Resistenz letzten Endes nur ein besonders auffallender Teil der vitalen ist. Das zeigt sich, zweitens, auch darin, dass der mehr oder weniger inaktive Ruhezustand ausbzw. angetrockneter Thalli bei der Wiederanfeuchtung keineswegs unvermittelt in die normale frühere Vitalität übergeht (vgl. schon MUENSCHER 1915). Vielmehr zeigt die zunächst eintretende Steigerung der Atmung und Depression der Assimilation, dass die Wiederherstellung eines voll aktiven Plasmazustandes Energie und Zeit benötigt, und das umsomehr, je stäker die An- bzw. Austrocknung war und je länger sie gedauert hat. (Algen : MONTFORT 1937 ; Flechten : STÅLFELT 1938 ; ENSGRABER 1952 ; LANGE 1953 ; PLANTEFOL 1927 ; STÅLFELT 1937 ; ROMOSE 1940 ; Lebermoose : ENSGRABER 1952 ; Farne und höhere Pflanzen : MONTFORT u. HAHN 1950.) Vorhergegangene Abhärtung, aber auch Verweichlichung spielen bei der Geschwindigkeit der Erholung eine Rolle (ENSGRABER). Die Schädigung beim Aus-bzw. Antrocknen ist für die einzelnen Lebensvorgänge verschieden stark und hat deshalb vermutlich mehrere Angriffsstellen am Plasma ; besonders empfindlich ist die Photosynthese, und es kann vorkommen, dass bei der Antrocknung zwar die Vitalität an sich erhalten geblieben, die photosynthetische Fähigkeit aber so stark geschädigt worden ist, dass sie beim Wiederanfeuchten entweder ausbleibt oder nach einem kümmerlichen Erholungsanlauf irreversibel schwindet und damit dann den Thallus nach einiger Zeit zum Absterben bringt (ENSGRABER). Es scheint mir bemerkenswert, dass in der auf *Hitze*-Inaktivierung folgenden Erholung die Reaktivierung der Photosynthese bei dem von ROMOSE untersuchten Laubmoos einen anderen Verlauf nimmt als nach Austrocknung ; das spricht gegen die von LEVITT (1951) postulierte *Gleichheit* des plasmatischen Geschehens bei Trocken-, Hitze- und Frostresistenz.

Die sich im Ablauf der Erholungsvorgänge ausdrückende « *Dynamik* » der letalen Plasmaresistenz ist *ökologisch* höchst bedeutungsvoll (MONTFORT 1937 ; MONTFORT u. HAHN 1950). Dabei muss die genotypische Veranlagung in Ubereinstimmung mit den ökologischen Anforderungen des Standortes sein (vgl. BIEBL 1949), aber nur insoweit als diese lebenbegrenzend sind. Im übrigen kann sie sich frei entfalten, und so kann es vorkommen, dass extrem hygrophile Arten volle Austrocknungsresistenz besitzen, die ökologisch nicht beansprucht wird, sondern nur durch die systematische Verwandtschaft genotypisch bedingt ist, wie etwa bei der Hymenophyllacee *Trichomanes radicans* (MONTFORT u. HAHN). Auch bei den Flechten scheint die genotypische Austrocknungsresistenz sowohl des Pilzes wie der Alge eine grosse Reserve gegenüber allen Standortsansprüchen zu bieten, im Gegensatz zu der letalen Hitzeresistenz (LANGE).

Bei den *Kormophyten* tritt die letale Resistenz gegenüber der vitalen an ökologischer Bedeutung zurück. Man kann sie auf das jeniger Sättigungsdefizit der Pflanze beziehen, von dem ab bei Wiederzufuhr von Wasser keine Erholung mehr eintritt, und wenn man damit das am Standort in Trockenzeiten erreichte Defizit vergleicht, kommt man zu einem Mass für die « *Beanspruchung* » *des Wasserhaushaltes* » (OPPENHEIMER, ROUSCHAL, HÖFLER-MIGSCH-ROTTENBURG). Okologisch entscheidender als diese Grössen ist aber die für den Kormophyten mögliche zeitliche Verzögerung ihres Eintritts, die *konstitutionell*, in erster Linie durch die artspezifische Spaltenreaktion und die Grösse der kutikularen Transpiration, bedingt ist (PISEK. u. BERGER, PISEK u. WINKLER).

b) Die vitale Plasmaresistenz

Die im *Verlauf* der Antrocknung sich abspielenden *vitalen* Vorgänge führen einerseits zu in einer « Ertragsminderung » gipfelnden « Dürreschäden », andererseits zu einer den Schäden entgegenwirkenden « *Abhärtung* ». Es ist von vornherein zu vermuten, dass in den beiden Fällen die Vorgänge einander entgegengesetzt verlaufen werden, indem ihnen das eine Mal eine *Des*organisation des Plasma zu Grunde liegt, das andere Mal aber eine *Re*organisation. Wenn wir beispielsweise die *Atmung* junger Haferpflanzen verfolgen, so finden wir sie beim Einsetzen und Fortschreiten einer Bodenaustrocknung stark erhöht, beim Stehenbleiben derselben aber auf ein Niveau absinken, das gegenüber dem Ausgangszustand umsomehr erniedrigt ist, je trockener jetzt der Böden gehalten wird (KRAFT 1944, vgl. STOCKER 1947). Ich habe diese beiden Phasen, die sich natürlich teilweise überdecken, in Analogie zu den Reizvorgängen als « Reaktions » - und « Restitutionsphase » bezeichnet (STOCKER 1947, 1948). Es ist ein verhängnisvoller Fehler, dass man dieser zeitlichen Dynamik der Trockenwirkung bisher keine Beachtung geschenkt hat, sondern der Meinung war, dass die Dürreeffekte in einer geraden Linie zur Abhärtung führen und deshalb gleichsinnig zu jedem Zeitpunkt erfasst werden können ; auch das Referat von LEVITT (1951) steht offenbar noch auf diesem Standpunkt. Die Folge ist, dass sich manche Ergebnisse verschiedener Autoren widersprechen, weil sie in phasenmässig nicht unterschiedenen Zeitpunkten gewonnen wurden. Wenn wir eine Förderung gegenüber dem Ausgangswert mit +, eine Hemmung mit — und Unsicherheit in der Zuordnung zu der betreffenden Phase mit ? bezeichnen, so ergibt sich für einige einigermassen übersehbare Dürreeffekte das folgende Bild :

Effekt	Reaktionsphase	Restitutionsphase
Permeabilität (Harnstoff)	? + 1)	— 2)
Viskosität	? — 3)	+ 2)
Atmung	+ 4)	— 5)
Assimilation	— 6)	+ 7)

1) Levitt and Scarth (1936). — Whiteside (1942). — 2) Schmidt (1939). — Schmidt, Diwald u. Stocker (1940). — 3) Northen (1943). — 4) Kraft (1944). — Ensgraber (1952). — 5) Kraft (1944). — 6) Ensgraber (1952). — 7) Simonis (1947).

In Verbindung mit der Tatsache, dass *Schüttelbewegungen* eine Reaktionsphase mit denselben, offenbar auf thixotropen Lockerungen der kolloidalen Eiweisstruktur beruhenden Effekten ergeben (Kahl 1951) ist von mir eine *Theorie der plasmatischen Dürreresistenz* zur Diskussion gestellt worden (Stocker 1948). Sie geht von der Annahme aus, das bei fortschreitender Dürre der Wasserentzug Zerreissungen und Lockerungen des Eiweissmolekül-Netzwerkes des Plasmas zur Folge hat. Das bedingt in der Reaktionsphase einmal Anderungen der strukturellen Grössen wie Permeabilität und Viskosität, andererseits Beeinflussungen der Fermentreaktionen, die zu einer weitgehenden Umsteuerung des Stoffwechsels von der reduktiv-aufbauenden zur oxydativ-abbauenden Tendenz führen, besonders ausgeprägt bei Assimilation und Atmung. In der Folge dieser Vorgänge entstehen dann neue Bedingungen, die zu einer Wiederknüpfung von Haftstellen des Plasmagerüstes führen. Indem die damit eingeleiteten *Restitutions*-vorgänge noch über den Ausgangszustand zurückpendeln, entsteht die zusätzlich stabile *Abhärtungsstruktur*. Für die letale *Hitzeresistenz* macht Bogen ebenfalls eine kolloidale Störung des Eiweissmolekül-Netzwerkes verantwortlich, die auch ganz im Rahmen der modernen Protoplasmatik liegt. Wenn Levitt in seinem Referat (1951) unsere Vorstellungen ablehnt, so hat er letzthin als Argument, dass Frost-. Trocken- und Hitzeresistenz alle auf gleicher Basis beruhten und durch eine einzige Theorie, die seinen Frostuntersuchungen nicht widersprechen dürfe, erklärt werden müssten. Dass diese bei so verschiedenartigen Einwirkungen von vornherein unwahrscheinliche Ansicht nicht haltbar ist, geht u. a. daraus hervor, dass bei Getreide die Kälteeffekte sowohl in der Reaktions- wie in der Restitutionsphase genau entgegengesetzt den Trockeneffekten verlaufen (Andersòn 1944).

III. Die konstitutionelle Trockenresistenz

a) Thallophyten

Die *hydrolabile* Konstitution der *Thallophyten* gibt nur geringe Möglichkeiten einer Unterstützung der plasmastichen Resistenz. Am ehesten ist das bei der *vitalen* Resistenz möglich, indem etwa bei den Fucaceen des Litorals die hohe Quellungskapazität des Thallus die Austrocknung verlangsamt und damit die Assimilationsperiode bei Ebbe verlängert (STOCKER u. HOLDHEIDE 1937).

b) Kormophyten

Beim *Kormophyten* hat die *hydrostabile* Konstitution entscheidende physiologische und ökologische Bedeutung. Ihren Einfluss auf die *letale* Resistenz haben wir schon erwähnt. Noch wichtiger ist die Stabilisierung der *vitalen* Produktion, die, wenn wir an die Eroberung der Trockengebiete durch die höhere Pflanzenwelt denken, geradezu den Kernpunkt des Xeriphytenproblems bildet.

Die Behandlung des Xerophytenproblems ging bekanntlich von den auffallenden *morphologischen* und *anatomischen* Strukturänderungen aus, welche unter Trockenbedingungen phäno- und genotypisch auftreten. Die weitgehenden Schlussfolgerungen freilich, die man seit SCHIMPER und WARMING aus diesen *Xeromorphosen* ziehen zu können glaubte, müssen heute nach zwei Richtungen hin kritisch revidiert werden : Einmal steht es nicht fest, inwieweit sie *direkte* Trockenwirkungen sind, da gewisse Xeromorphosen auch in Feuchtkultur durch Stickstoffmangel erzeugt werden können (MOTHES, MÜLLER-STOLL, SIMONIS 1948). Zum anderen hat die experimentelle Analyse schwere Irrtümer aufgedeckt, die durch eine nur *deduktive* Ableitung physiologische Effekte aus morphologischen Strukturen entstanden waren, so z. B. die Annahme einer dauernd eingeschränkten Transpiration der Xerophyten (siehe z. B. MAXIMOW). Damit drängt das Problem der Trockenresistenz auf eine möglichst umfassende Erforschung der *artspezifischen Konstitution* hin. Sie ist zunächst als Produkt von genetischen Entwicklungstendenzen innerhalb der systematischen Verwandschaftskreise zu betrachten, in dem die Selektion nur das entscheidend lebensfeindliche ausgemerzt hat. Auf dieser Grundlage ist dann festzustellen, was der Trockenresistenz nachweisbar dienlich ist (ECKARDT 1952).

Um den *genotypischen* Faktor möglichst auf die für die Dürreresistenz verantwortlichen Gene zu beschränken, arbeitet man am besten mit *dürreresistenten* und *dürreempfindlichen Rassen* ein und derselben Art, und lässt gleichzeitig den *phänotypischen* Faktor in *Trocken-* und *Feuchtkulturen* in Erscheinung treten (SCHMIDT, DIWALD u. STOCKER 1940, STOCKER, REHM u. SCHMIDT 1943). Man erkennt dann als gemeinsamen Zug der konstitutionnellen Trockenresistenz die Tendenz zur *Stabilisierung* — nicht zur Einschränkung — des *Wasserhaushaltes* und damit des *Assimilationshaushaltes*. Dieses Ziel wird aber im Einzelnen auf sehr verschiedene Weise und mit sehr verschiedenen Mitteln angestrebt. Es schälen sich Konstitutions*typen* heraus, die man in erster Annäherung als stabile und labile, träge und lebhafte unterscheiden kann (STOCKER 1937), wobei aber, wie bei der Zuckerrübe, ein sehr labiler Wasserhaushalt mit einem ziemlich stabilen Assimilationshaushalt kombiniert sein kann (STOCKER, REHM u. SCHMIDT).

Um an den *Trockenstandorten* selbst expermentelle Konstitutionsforschung treiben zu können, mussten zunächst feldmässig brauchbare *Methoden* geschaffen werden. Zu den meist gebrauchten gehören die Kryoskopie von. Pressäften zur Hydraturbestimmung (WALTER 1931) und die Momentanmethoden zur Messung der Transpiration (STOCKER 1929) und der Assimilation und Atmung (HOLDHEIDE, HUBER, STOCKER 1936, jetzt erheblich verbessert). Andere wichtige Vorgänge, vor allem die Wasseraufnahme harren aber noch branchbarer Bestimmungsmethoden ; für die Saugkraft eröffnet vielleicht die Refraktometrie gute Aussichten (LEMÉE u. LAISNÉ 1951). Da bei dem Wechsel der Klima- und Bodenbedingungen von Tag zu Tag nur gleichzeitige Messungen der verschiedenen physiologischen Zustandsgrössen und Reaktionsabläufe zur Erkennung des Konstitutionsbildes brauchbar sind, erfordert die moderne Feldökologie eine *Gemeinschaftsarbeit* von Spezialisten. Die Menge und der Umfang der dabei zu transportierenden Apparate verlangt ein motorisiertes Laboratorium, womit gleichzeitig der Aktionsradius und die Wahl geeigneter Versuchsstellen sehr gefördert wird. Nach diesen Uberlegungen war unsere letzte grosse Unternehmung in die südalgerische Sahara organisiert, wobei wir uns des « Laboratoire de Biologie Saharienne » (KILLIAN 1940) in Beni Ounif als Stützpunkt bedienen durften ; dafür möchten wir seinem um die ökologische Forschung so verdienten Direktor, Herrn Prof. Dr. KILLIAN, auch an dieser Stelle unseren wärmsten Dank zum Ausdruck bringen.

Das aus allen Erdteilen vorliegende sehr umfangreiche Material über die *Okologie der Trockenpflanzen* auch nur in seinen wichtigsten Arbeiten zu zitieren, ist hier unmöglich. Mehr und mehr hat sich gezeigt, dass *konstitutionelle Trockenresistenz* auf sehr verschiedene Weise erreicht wird. Dabei werden aber keine spezifisch neuen morphologischen oder physiologischen Eigenschaften entwickelt, sondern die Resistenz wird durch Steigerung und Kombination *allgemein* gegebener *Potenzen* erreicht. So treffen wir einerseits an jedem Trockenstandort ein Gemisch verschiedener, ökologisch möglicher Konstitutionen, und andererseits in den einzelnen Florenreichen auffallende Xerophytenformen aus genotypisch bestimmten und florengeschichtlich gewordenen Verwandtschaftskreisen, wie etwa in Zentralamerika die Kakteen, in Makaronesien die Euphorbien und in Südafrika die Mesembryanthemen.

Die viel umstrittene Frage KAMMERLINGS (1914) : « Welche Pflanzen sollen wie *Xerophyten* nennen ? » scheint mir heute überholt zu sein. Die einzelnen Typen der Trockenstandorte bewohnenden Pflanzen, die man etwa in Therophyten, Geophyten, Hartlaubpflanzen, Grundwasserpflanzen, Sukkulente und « echte Xerophyten » im Sinne Maximow's unterscheiden kann, haben sich als in sich so uneinheitlich und unter sich durch so viele Uebergänge und Ueberschneidungen verbunden erwiesen, dass man das Xerophytenproblem nur in seiner *Gesamtheit* erfassen kann ; « anuelle Xerophyten » (KILLIAN 1942) und « mesomorphe Xerophyten » (ECKARDT 1953) seien als zwei Beispiele unter vielen genannt.

Eine *umfassende* Betrachtungsweise erfordert auch die autökologische *Konstitutionsuntersuchung.* Neben Lebensweise der ausgewachsenen Pflanze muss in gleicher Weise ihre *Entwicklungsgeschichte* studiert werden, die auch bei Perennen von entscheidender Bedeutung sein kann, so vor allem bei der Samenkeimung und dem Keimlingswachstum (z. B. Killian 1939 u. 1949, Went 1949). Die Untersuchungsarbeit an der *ausgewachsenen Pflanze* ist vielseitiger und dynamischer geworden. So haben z. B. Pisek u. Tranquillini an der Fichte die *jahreszeitliche* Verlagerung der Transpiration zwischen Basis und Gipfel und Sonnen- und Schattenseite verfolgt. Aber die *Transpiration* allein (z. B. Stocker 1931, 1933, 1935) gibt kein Mass für den Waserhaushalt im ganzen, ebensowenig wie die *kryoskopisch* gemessenen *osmotischen Werte* (Walter 1931, 1949). Es fehlt uns noch an Methoden, die im Rahmen von Tagesserien Messungen der Saugkraft, der Wasseraufnahme und anderer wichtiger Grössen erlauben.

Gerade die *Wasseraufnahme* der Xerophyten bedarf dringend einer differenzierenden Untersuchung, auch hinsichtlich der Mikroverhältnisse des Bodens. Hier ist der Nachweis wichtig, dass das *Tensiometer* nicht die *Bodensaugkraft,* sondern viel geringere Drucke in einem steilen Feuchtigkeitsgefälle angibt, das sich infolge der sehr geringen *Wassernachleitfähigkeit* trockenen Bodens um die Tonzelle herum einstellt (Stocker u. Kausch 1952, Kausch 1953). Physiologisch erwächst aus der Langsamkeit der Wassernachleitung die Notwendigkeit, dass die Wurzeln dem Wasser nachwachsen. Daraus entsteht die ökologische Frage, ob nicht die dürreresistenten Pflanzen durch eine trockenbedingte Förderung des Längenwachstums der Wurzeln konstitutionell ausgezeichnet sind (Kausch 1953 und 1954 noch nicht publiziert) ; damit würde die Schnelligkeit der Wurzelneubildung nach Niederschlägen eine wichtige ökologische Ergänzung erfahren.

Eine grosse Rolle in der heutigen Forschung spielt das Verhältnis zwischen Wasser- und *Assimilathaushalt.* Die jetzt verfeinerten Methoden ergeben, dass die Beziehung : Spaltenweite ± → Transpiration ± → Assimilation ± keineswegs immer gilt. Es gibt Fälle, wo bei noch guter Spaltöffnung und merklicher Transpiration die Assimilation längst zusammengebrochen ist (Stocker u. Mitarb., noch nicht publiziert), oder wo Transpiration und Assimilation wenig beeinflusst werden durch sehr starke Anstiege des Sättigungsdefizites (Eckardt 1953). Eine notwendige Übereinstimmung von Wasser- und Assimilationshaushalt besteht weder hinsichtlich der Stabilität oder Labilität noch hinsichtlich des Intensitätsspiegels. Sicher scheint, dass innerhalb der konstitutionellen die plasmatische Resistenz der Photosynthese eine Rolle spielt. Konstitutionelle Zusammenhänge der Assmilation mit Blattgrösse und Länge des Wurzelwerkes deutet Eckardt an.

Diese Ausweitung im Gebiet der Photosynthese ist symptomatisch für die *weitere Entwicklung* des Dürreresistenz-Problemes. Es wird sich noch erheblich verbreiter müssen : Einige Richtungen sind schon sichtbar, wie etwa Mineralsalzhaushalt, Stickstoffhaushalt, Wachstum und Differenzierung. Ein grosses Feld künftiger Forschung steht offen.

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THE DESICCATION OF PLANT ORGANS

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There has been inceasingly more and more published about dehydration. The theoretical foundations of the drying and desiccation of biological material have not yet been investigated in detail. It is very difficult to follow in detail and continuously the loss of water in drying biological material. If we wish to consider the smallest details it is almost impossible to determine the losses of weight on an analytical balance. Pr. Dr. St. ŠKRAMOVSKY constructed an apparatus which records on the basis of photoregistration the whole course of the loss of water quite automatically in graphs. On the horizontal axis the time and on the vertical axis the decrease in weight is marked. ŠKRAMOVSKY called the apparatus stathmograph.

The apparatus makes it possible to work both with a constant temperature and with a steadily and uniformly rising (increasing) temperature. Here the proportionate speed of the automatically switchedin registration correspond to the different speed of the rising temperature. The results of the tests made in the years 1934 and in 1949, 1950 and 1953 agreed well so that there is no need to give them separately.

In order to make the samples as equal as possible we cut a piece from the middle part of the leaf blade, on both sides of the main rib.

When no special questions were concerned, the leaves were as far as possible equally developed, *i. e.* mature.

TEST WITH A CONSTANT TEMPERATURE

Leaves

The curves obtained in drying under constant temperature are relatively simple. The loss of water runs regularly in a smooth curve with a greater or lesser initial gradient, and with a gentle or sharper bend it passes into the horizontal of the constant weight. The shape of the curve depends especially on the plant material, on its structure and on the height of the temperature used.

At temperatures around 20°C the plants lose water very slowly and the graphs indicate a progressive, very gradual decline, and on the whole a linear dependence on time. The leaves of *Tradescantia* do not attain constant weight even after 12 days. The apparatus offers many advantages for these type of measurements, but it has not as yet been fully used for them.

When we calculate the time from the beginning of the drying to the moment when the leaf attains a constant weight at higher temperatures, we find that the dependence is not linear at all for various temperature. Whereas at temperatures up to + 40 to + 50°C a relatively small increase of temperature has a very considerably influence, the loss of water is accelerated only very little by an increase of the temperature from a certain point which we can designate as critical temperature. Sometimes it is striking that in a certain interval of temperature the speed of drying changes very little. Only considerably higher temperatures cause again a more rapid loss of water.

Model Tests

In order to contribute at least somewhat to the knowledge of the fundamental physico-chemical factors we carried out different control tests on the evaporation of water. Water or solutions soaked into a strip of filter paper were the simplest control, which hung up into the drying oven was dried similarly as the part of the leaf. In simple compounds the curves differ from the curves of the leaf. A striking difference was not only the quicker loss of water from the paper, but especially also the much sharper break previous to the constant weight of the dry matter. This applies to the solutions of natrium-chloride as well as to the solutions of saccharosis. The yolk of an egg, on the contrary, gave curves much more like the curves of dried leaves. The gradient runs from the beginning in a distinctly concave curve, and passes gradually without a distinct break into the part parallel to the axis X (corresponding to the dry matter).

TESTS AT INCREASING TEMPERATURE

The process of the loss of water at a temperature gradually and equally rising is of course much more complicated and considerably more varied than when plants are dried at a constant temperature. The graphs are more complicated, and the curves show a different course, either uniformly continuous or with various bends and waves or breaks.

Most of the tests were made so that the time of a rotation (revolution) of the registration drum was chosen as for 6 hours ; during this time the temperature rose by 96°C, *i. e.* the increase of temperature was always 8°C in 30 minutes.

When the temperature rises equally, the resultant curves have certain characteristic features. Most distinct are the breaks which arise when at the beginning the weight decreases slowly ; at a certain temperature, however, the water begins suddenly to evaporate more rapidly and the curve descends more steeply. This wave takes place at a certain temperature and not after a certain time. Also the amount of water evaporated is not of so great importance. Thus in the plant *Zebrina pendula* the maximum decline is around 65°C, in *Pelargonium zonale* around 55°C.

A considerable influence of damaging or killing substances manifests itself also at an increased temperature. The influence of freezing in leaves of *Pelargonium zonale* was followed in greater details. Two surprising phenomena were observed here : 1. The curve of leaves frozen at a temperature slightly under zero differs relatively little from the graphs of living leaves. 2. Leaves frozen at a temperature of — 30° or — 70°C have a different curve and differ distinctly also from leaves frozen in liquid air (—190°C).

In a further series of tests the samples from different parts of leaves or from leaves of different age were observed. A rather detailed analysis of the curves shows : the curves which result when different samples are dried are different ; yet they agree in showing waves at certain temperatures equal in all curves. Sometimes the waves are very distinct, so that they determine the character of the whole curve ; but in other cases only in a detailed analysis the waves can be stated. Thus e. g. in the leaves of *Tradescantia albiflora* in the youngest leaves there is a distinct break around 45°C ; in older leaves there is at about the same temperature a hardly discernible wave. At a temperature of 30°C there is, on the contrary, in older leaves a distinct break, whereas for younger leaves we do not see it in the original graphs and can observe it only by detailed analysis. Also the temperature at which all the samples lose water most quickly, agrees much. It lies between 55° and 60°C.

It is remarkable how young leaves of *Tradescantia, Callisia, Camellia* lose especially at the beginning water more slowly than fully developed leaves, even though the whole time of drying may be a little shorter. The relation of the curves at a rising temperature and at a constant temperature is not simple. A leaf with stalk of *Tradescantia* in a weight ratio of 3 : 1 dried at a temperature of

42°C in	14 1/2 hours,	
45°	in about the same time,	at a temperature of
47°	in 7 hours,	at a temperature of
51°	in 6 1/2 hours,	at
52°	in 5 hours,	at
53°	3 1/2 hours,	at
55°	in 3 hours,	at
58°	in 3 hours.	

It can be observed that at a temperature of 48°C the time of drying was considerably reduced. It is the same temperature where with the rising temperature a break occurs in the graph.

With the temperature also the resistency of the plant to loss of water is changed. Cuts of the plant *Tradescantia albiflora* were placed in the drying oven where the samples were weighted. The weight ratio of the leaves to the stalk was on an average 3 : 1. After a certain time we removed from the drying oven always one plant, placed it in water and left it in good conditions. From the graphs it could be approximately stated how much water the plant had lost at the moment when it was taken out. While at temperatures below the break the plant tolerates a loss of 55 till 60 % of its weight, at higher temperatures, *i. e.* at those lying above the break, it dies already at a slight loss of water. At 51°C it dies at a loss of 60 % of its weight. At higher temperatures it dies already with the loss of 40 and 30 %. *Zebrina pendula* behaved similarly at 53°C and 59°C, *i. e.* again approximately at the break indicated in the graphs of this plant.

The drying curves of the stems are often considerably simpler than those of the leaves. As far as we can follow the breaks here, they are less distinct than in the leaves. Specially simple are the graphs of homogeneous parenchymatic tissues. The influence of the thickness of the part cut-out is of course very clear.

We carried out a series of model tests also with the rising temperature. There was again a very distinct difference between simple solutions and colloids. Gummi arabicum with a quick initial gradient and a relatively sharp bend differs from the elongated concave graphs of the albumins, which lose water much more slowly, and whose graph passes without any break gradually into the horizontal expressing dry matter.

The control tests entitle us to draw the conclusion that from the tissue the water does not evaporate simply as from a free water level or from a watery solution of simple substance, *i. e.* salts or simple colloids. It holds even for dead tissues. The beginning of the curve is always gradual and the end passes regularly gradually and gently into the horizontal, as we observed it in solutions especially of albumins. We have here to do with water bound more firmly than osmotically even in dead tissues.

The answer to the question whether living or dead leaves dry more quickly cannot be answered uniformly. It depends not only on the species of plant and on the age of the leaf, but also on the method of killing. And the temperature is also a decisive factor. At ordinary temperature dead leaves usually dry much more quickly than living ones, but at increased temperature the change of structure (ultrastructure) causes sometimes the loss of water from the killed leaves to be considerably slower, and the process of drying changes. This refers to the whole time necessary for the tissue to dry as well as to the whole course of the loss of water shown by the shape of the curve. The colloid structures change under the influence of the killing means as well as under the influence of temperature. Changes in the course of the curve are correlated to these factors.

In studying the rising temperatures the bends or breaks which appear more or less distinctly at a certain temperature are important. They have a relation to the course of the curve registered at a constant temperature. This seems to indicate that at the temperature of the bend the cells die or at least the permeability for water is disturbed and the relation of bound water is changed. The fixation of water is changed under these conditions.

F. **Franek** tried to find a mathematical expression of the drying curves ; his most important conclusion is that in the drying of potato cuts at no temperature and at no thickness of the cuts used, the function is of simple exponential type. To a visual examination the graphs at low and at high temperatures may seem qualitatively different, especially by the sudden change at high temperatures ; but the type of the curves is the same and their constants change with the temperature. These questions will be discussed in a later paper.

The stathmographic apparatus may be useful also in other spheres. Thus e. g. the drying curves of thermophile *Cyanophyceae* (*Mastigocladus, Symploca*) run smoothly without breaks or waves. Some succulents (e. g. *Sedum, Sempervivum*) grow in localities whose substratum is heated through by the sun ; in the morning they are exposed to gradually rising temperature which easily exceeds 40°C. Thus it is possible to use this method also in solving similar physiological and ecological questions.

EXAMPLES OF ROOT ADAPTABILITY TO ARIDITY

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There are many different ways in which various plant species achieve their necessary water balance in arid regions. Of particular interest is the phenomenon of specific adaptability of the root systems of many species. Two examples may serve to elucidate this : the first is based on the investigations of the senior author and the second on those of A. Abraham.

I. *Artemisia herba alba* Asso. may be taken as the first example being, also, of plantgeographical importance. Its macro-distribution stretches from Morocco, through the Sinai peninsula and the Negev in Israel, deep into the Turanean region, namely Afghanistan. In this whole region it thrives however only in the strip between the 150 to 400 mm isohyetals, with cool to cold winters and sometimes covered by snow. The latter seems to stimulate germination so that the distribution of *A. herba alba* seems to indicate the area of occasional snow fall.

According to our observations, the young plant develops a tap root of 10 to 15 cm during the first year. From the second or third year, however, the development of the root system varies with different ecoclimatic and soil conditions :

At the border of its distribution area, *i. e.* at about 150 mm average annual rainfall (if we take this as the indicator of the degree of aridity), or on loess soil, where penetration is very slow and difficult, branching begins already at 2 to 5 cm depth. This is obviously due to the fact that even relatively heavy rains do not penetrate into a deeper layer unless they occur at short intervals, which is rarely the case in this area. The deeper part of this young tap root dries out and dies.

However in regions with higher amounts of rain and on hard limestone with its vertical crevices, which serve as water pockets weeks after a rainfall, the tap root grows downward to 40 to 50 cm, branching only then. Where the soil from a more or less flat rocky layer has been washed away by one or more torrents, it is quite striking to observe the upper parts of these tap roots exposed up to 30 cm above the rock surface. Sometimes many hundreds of such *A. h. a.* individuals are to be found with their exposed roots all pointing in the same direction and with the bushes on the top end erect again and living. Of course the deeper parts of the root systems remain undisturbed in the crevices.

In spite of the high adaptability of its root system to the degree of aridity, the total ecological amplitude of *A. h. a.* is very small. This can be seen in its distinct distribution pattern which follows geological and soil conditions and the IE-factor, *i. e.* Insolation-Exposure conditions. In the much drier habitat of soft chalk (Mergel) in the Negev the species begins to occur only at an altitude 250 m above that on hard limestone, or at about 700 m as compared with 450 m above sea level. The « climatic soil coefficient » has to be regarded as a variable constant, to be determined empirically. In our region this climatic soil coefficient is minus 30 mm average yearly precipitation for sand, and plus 25 mm for soft chalk (Mergel), if we take hard limestone as the normal.

These determining distribution factors are of particular importance if plantecological methods are to be used to determine quantitatively the average precipitation in arid regions on the basis of the geoecological law as shown by this author (see Unesco-Turkey Symposium on Hydrology of arid zones, Ankara, 1952, Proceedings).

II. The second example shows another way of natural adaptation to extreme drought conditions.

The *Amaranthus blitoides* is an annual species which grows by the side of streets and on loose soil-mounds.

A very characteristic feature of this plant is its shallow root system and the small area taken up by the roots.

Although the plant grows in habitats which can be designated as dry, it grows and develops well in summer, which is the dry season in the Mediterranean area. It germinates in April-May and continues to develop and grow up to the first frost-wave of the coming cold season.

At the time of germination the conditions of soil moisture are favourable ; but already at the beginning of July the water conditions around the roots are near the wilting point and these conditions continue throughout its further growth.

The transpiration of the plant has been measured at various habitats and various stages of development. The evepotranspiration of the whole soil of the root area has been compared with the transpiration of the whole plant according to Huber.

It appears that the maximum available water would enable the growp-up plant to live only for 4 days. However, the available water in the soil at later dates (after mid-July) showed that there was enough water for only one day at the plant's disposal, or even less than that. The details of this work will be published elsewhere.

In this work it has been shown and proved that there is a direct connection between the available water in the soil and the growth of the plant. It has been proved that all the plants pass through a crisis in August and those which pass this test develop well in the late summer and early autumn, in spite of the fact that there are still no rains in this season. This period, however, excells in many dew days. But, according to the meteorological measurements of Dr. Ashbel, it appears that the dew alone cannot be responsible for the necessary amount of water for this plant because of frequent pauses, up to ten days, in the occurrence of dew.

A model was, therefore, built of the habitat of the plant and, with its help it was proved that the phenomenon of internal dew (sub-terranean dew) exists in this special habitat. According to the rootsystem of *A. blitoides* it must be concluded that it spreads just in his layer, where maximum condensation exists.

This gives us the only explanation for the surviving and evengrowing of this species in this season of the year.

LA RESISTANCE DU PROTOPLASME A LA SECHERESSE

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La résistance des plantes à la sécheresse est une qualité physiologique très complexe, résultant de la somme des éléments constitutifs qui permettent à la plante de survivre dans les endroits arides et de supporter de longues périodes de sécheresse. Un de ces éléments est la résistance au dessèchement. On peut la mesurer à l'aide d'une méthode de physiologie cellulaire, en déterminant le degré de réduction de l'humidité atmosphérique qu'une cellule, exposée à l'atmosphère, peut supporter encore. Jusqu'alors on était d'avis que cette résistance variable dépend de la capacité inégale du protoplasma à supporter le dégonflement. Mais Iljin put prouver d'une manière concluante que les cellules des plantes supérieures périssent quand elles sèchent, pour la plupart, par la rupture du protoplasme. En perdant son eau, la vacuole se rétrécit, entraîne le plasma et le détache de la paroi cellulaire au moment où celle-ci ne peut plus se rétrécir.

Lorsque ces cellules ont été plasmolysées avant, elles supportent un degré beaucoup plus haut de desséchement. Le revêtement périphérique du plasma se rompt souvent après avoir été humecté. Cet effet se produit, quand la membrane, s'étant rétrécie auparavant, se dilate subitement. En ces deux cas, il s'agit d'une mort mécanique du protoplasma par rupture.

L'antithèse de ce processus est la mort du protoplasme causée par le dégonflement, c'est-à-dire à la suite du desséchement du protoplasme même. En certains cas, on peut prouver que le dégonflement dans l'air sec est la cause de la mort du plasma et non le détachement de la paroi. Cela fut prouvé il y a quelques années pour les Hépatiques à feuilles (Jungermanniales). Dans leur cas, on peut exactement déterminer les limites vitales par des expériences en séries dans de petits exsiccateurs, au-dessus de H_2SO_4 de concentration croissante. Il n'y a pas seulement des espèces capables de se dessécher ou bien des espèces sensibles à la sécheresse, mais les limites vitales s'échelonnent en longues séries. Ainsi les limites sont pour *Aplozia riparia* à peu près à 90 % d'humidité atmosphérique relative, pour *Calypogeia Neesiana* de 85 à 80 %, pour *Trichocolea tomentella* de 70 %, pour *Bazzania trilobata* de 50 % d'humidité atmosphérique (valeurs relatives à 20° C.), pour *Madotheca platyphylla* à 0 % environ, car les cellules de cette espèce survivent régulièrement au-dessus d'une solution concentrée de H_2SO_4. Les feuilles desséchées formant une seule couche ressemblent alors aux rayons de miel, car les parois cellulaires s'enfoncent profondément. Mais le plasma n'est pas endommagé, ni au cours du desséchement, ni au cours de la réhumidification. Les limites de résistance, variables d'espèce en espèce, sont déterminées par la pression de la vapeur à laquelle le plasma est exposé. Les valeurs-limites constatées dans les expériences en séries sont assez prononcées ; en cas de répétition elles sont identiques pour chaque espèce. Pour certaines espèces, les limites vitales peuvent être reculées par endurcissement (c'est-à-dire par traitement préliminaire dans les exsiccateurs sur H_2SO_4 de faible concentration), mais seulement jusqu'à une limite maximale qui paraît être fixée par la constitution du protoplasme de l'espèce.

Il est intéressant d'examiner les protoplasmes des Hépatiques séchées partiellement mais tout à fait vivantes, en rapport avec différentes qualités comme p. e. la perméabilité aux substances dissoutes à petites ou grandes molécules, ou l'aptitude à la coloration vitale. La valeur osmotique des cellules (Will-Richter) reste la même après l'humectation ainsi qu'avant le desséchement. Il est sans importance que les sucs cellulaires séchés à l'air aient été épaissis et les substances du contenu précipitées.

Pour les Mousses (Musci), la détermination de la limite du desséchement est déjà plus difficile, car les deux causes discutées peuvent être déterminantes, c'est-à-dire qu'il pourrait s'agir d'une mort mécanique par rupture ou bien d'une mort causée par le desséchement du protoplasme même. Wo. Abel s'occupe de cette analyse dans notre institut à Vienne. J'ai

seulement étudié exactement *Hookeria lucens.* La limite vitale fixée par une simple expérience en série se trouve ici à 80-70 % d'hum. rel. Mais dans les exsiccateurs plus secs les cellules ne sont pas mortes, elles ne périssent d'une mort mécanique qu'après avoir été humectées. On peut éviter cela (d'après Iljin) en transportant les feuilles sèches d'abord dans une solution sucrée hypertonique. Alors la valeur-limite pour la mort plasmatique par la sécheresse est égale à 48 % d'hum. rel.

La détermination des limites vitales des plasmes à la sécheresse est une tâche importante de la protoplasmatique comparative. Dans toutes les théories sur la structure protoplasmique, il ne faut pas perdre de vue que les limites du desséchement, où la structure du plasma s'effondre, sont très différentes chez les diverses espèces de plantes et les sortes de cellules.

THE PART PLAYED BY STOMATA IN RESISTANCE TO DROUGHT

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If the stomata can in fact control appreciably the exchange of gases between leaves and the external atmosphere they may assist survival of drought in two diametrically opposed ways. The more obvious is by closure when evaporating power is high ; the less obvious is by opening for at least part of the day (or perhaps the night in the case of succulents) when the conditions are favourable for CO_2 assimilation, possibly even in spite of severe drought. It is thus all too easy for the teleologist to find, in *qualitative* terms, a good reason for every stomatal movement and the need for quantitative investigation is paramount.

Milthorpe found that wheat stomata did control transpiration over the whole of the wide range of movement that he explored ; this was in moving dry air — the most important conditions for the present topic. It is thus worth considering what conditions cause stomatal closure. Wheat stomata tend to close with increasing CO_2 supply. In darkness, even a small increase above zero concentration causes almost complete closure and under natural conditions therefore this must always occur. In light (90 f. c. and upward) CO_2 has no effect between zero and 0.01 % ; of interest because 0.01 % approximates to the *minimum* in the intercellular spaces due to photosynthesis. Further increase to 0.03 % causes partial closure, which would thus occur with wind bringing fresh supplies of CO_2 to the leaf. The desiccating effect of wind would thus be partially prevented and although the closure would tend to restrict assimilation the steeper CO_2

gradient would act in the opposite sense. Closure due to CO_2 becomes less complete with increase of light (to 800 f. c.), implying less restriction of transpiration at high light when evaporating power is often also high. These experiments were, however, carried out with humid air. Wheat stomata also respond to changes in evaporating power of the surrounding air. It was found that increasing the flow of saturated CO_2-free air across the leaf from 2 to 12 1/2 1/hr. was without effect, but with dry air marked closure resulted. This would tend to occur in the dry conditions often found with high light. Whether such closure is partly due to direct water loss from the guard cells to the exterior or solely to general loss of leaf turgor is uncertain. In general, loss of leaf turgor causes stomatal closure ; preliminary opening probably only occurs if wilting is very rapid, as when the water supply to a detached leaf is removed. Leaf water content has no *direct* effect on transpiration, in the absence of stomatal control, until severe water loss has occurred (Gregory *et al.*) ; an indirect effect acting through stomatal closure thus appears biologically advantageous. Severe and rapid wilting causes the following after-effects on restoration of water supply to the leaf : failure to regain the pre-wilting water content, failure of the stomata to reopen fully and lower transpiration for a given stomatal aperture. The effect on transpiration is gradually reversible and appears due to changes in the water permeability of the transpiring cells.

If the plant is to survive prolonged drought the stomata must admit enough CO_2 for assimilation to exceed respiration. Maskell, and later Penman and Schofield, concluded that stomata only control assimilation when nearly closed, owing to a high aqueous diffusive resistance in the mesophyll cell and possibly a high « chemical resistance » at the chloroplast. If so it might be asked on teleological grounds why the stomata need ever open widely — perhaps in nature they seldom do. Maskell's results are open to some doubt because he used a permanently attached porometer cup and it is now known that under such enclosed conditions the stomata continue opening in the light until far wider than on the rest of the leaf where they are exposed to a constantly renewed CO_2 supply. Penman and Schofield assumed a gradient from 0.03 % CO_2 to zero instead of 0.03 % to 0.01 % ; thus the effect attributed to high aqueous diffusive resistance was at least in part due to the less steep gradient. Such a resistance, although expected on theoretical grounds, might be partly overcome by cytoplasmic streaming. The question of stomatal control of assimilation is thus still *sub judice* and is being investigated ; at least at the narrower apertures, however, there is a large measure of control. Pelargonium leaves in ordinary (0.03 %) moving air showed assimilation rising with stomatal opening until the air after passing over the leaf had only 0.01 % CO_2 content. Further opening of the stomata could not then increase assimilation as all the available CO_2 was being taken up.

It may be that such xerophytic characters as hairyness and deeply sunken stomata, by interposing a diffusive resistance between the guard cells and the external air, maintain relatively low CO_2 and high water vapour contents at the stomata and thus ensure sufficient stomatal opening. In *Pelargonium*, however, the stomata respond to the CO_2 content in the substomatal cavity and this would be but slightly affected by such added resistances unless they were very great ; if very great they would much restrict assimilation.

INFLUENCE OF WATER UPON ABSORPTION BY PLANTS OF ELEMENTS FROM THE SOIL

by W. S. Iljin

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The quantity of water in the soil influences absorption by the roots of salts whether moisture is insufficient in amount or whether it is in excess. Miller and Duley (1925), Emmert (1936), Thomas et al. (1942, 1943), and James (1948) observed in their experiments that the elements accumulate in plants when water is insufficient. But, on the contrary in certain cases, the lesser amounts of elements were absorbed from dry soils.

My observations made use both of plants grown under cultivation and of wild plants. In general those from dry sites contained more nitrogen than did those from moist sites. Determinations were made 4 times during the growing season, in the case of *Syringa vulgaris*, using the leaves only. It was found that the total nitrogen content of such plants, grown near Vienna, decreased from spring toward autumn, being 2,53 % in spring and 1,71 % in autumn in moist sites and 3,11 % in spring and 1,85 % in autumn in dry sites. Leaves from plants from the dry site had a slightly greater content of soluble nitrogen and amino acids. A similar decrease in total nitrogen was noted in the leaves of *Sambucus nigra*. The soluble nitrogen and amino acids of this species did not differ markedly in leaves from the two sites.

A large difference in total calcium and soluble calcium was found between leaves from plants of these two types of sites, particularly when the plants were grown on calcareous soils. Thus, the leaves of *Anthyllis vulneraria* contained 3,70 % total calcium, from plants growing on moist sites and 8,0 % on dry sites. Certain other species showed lesser differences in total calcium between leaves from plants from moist and dry sites, as for example *Sambucus nigra* (0,93 % and 1,44 %, respectively).

Very similar results were obtained in K content as in Ca content. Thus the K content of *Achillea millefolium* was 2,18 % fort a moist site and 2,92 % for a dry one in one instance and 2,76 and 4,96 %, respectively, in another instance.

Moisture content of the soil induces less change in magnesium content of plants, partly due to the fact that calcium may be substituted for magnesium by plants and partly because of the small amounts of magnesium that plants utilize.

In Venezuela, the author made observations on plants under cultivation and of naturally grown plants.

Sorghum was grown in the field on a silt loam and on a red lateritic soil. To the plots were applied different fertilizers compounded of superphosphate, ammonium sulphate, potassium nitrate, and potassium chroride. Each treatment was replicated 5 times. Because of excessive rainfall, water stood on some plots and the growth of sorghum on such plots remained significantly below normal. Samples for analysis were collected from 20 m^2 plots 62, 77, 98, 133, and 161 days after planting.

Normal plants attained a final height of 3-4 meters whereas those from affected areas were less than 2 meters tall. The dry weight yield per unit of land area had a ratio of 2-3 : 1 between normal and affected plants. During early stages of growth when the soil moisture was greatest the plants in the flooded sites had an average dry weight of 20,4 % and those of normal plants was 13,4 %, contrary to expectation. The variations in percentage of dry material at the end of 62 days were very large among the flooded plants but these differences were equalized as the vegetative mass continued to increase, or as growth proceeded.

Adventitious roots formed above the soil level, favoring absorption of water as shown by BERGMAN (1920) and KRAMER (1951).

Excess of moisture distinctly modified the physiological processes. This fact is shown by analysis made at the end of 62 days during which period of growth the plants suffered most from excess of water. The average percentage values from such analyses are presented in Table 1.

Table 1. Percentage of Materials in Normal and Affected Plants At The End of a Growth Period of 62 Days

	N	P	K	MG	Ca	Sugar & Starch	Hemicelluloses
Normal plants	1.85	0.241	2.24	0.160	0.277	3.3	11.2
Affected plants	1.19	0.215	1.67	0.067	0.217	5.7	14.5

As may be noted the amount of each of the elements absorbed was less in affected plants than in the normal ones but the former group contained the larger percentage of organic materials. The greater content of carbohydrates may be accounted for by decrease in consumption of carbohydrates and decrease of respiration under conditions where there was interference with absorption of minerals.

Although the growth of the plants was greatest during the early stages of development when rains were most abundant, conditions for growth improved later following the formation of adventitious roots. Such gradual improvement is shown by subsequent analyses of samples collected at later times, as indicated in Table 2.

Table 2. Changes in Amount of Absorbed Elements at Successive Periods

	Normal plants			Affected plants		
Sampling	K	Mg	Ca	K	Mg	Ca
First	2.24	0.160	0.277	I.67	0.067	0.217
Second	2.43	0.288	0.322	2.64	0.I75	0.256
Third	1.51	0.260	0.271	2.30	0.I73	0.256

It is shown that the absorption of magnesium was modified more by flooding than was that of Ca and K. Interference with absorption of Ca was greatest at first in flooded plants and there was less interference later, whereas the later samplings showed larger amounts of K in flooded plants than in normal plants. Probably the mechanism having to do with selective absorption by the protoplast of different elements is responsible for these differences.

The ability of the plant to utilize the elements contained in a unit area of soil may afford the best expression of mineral absorption.

In general flooded plants absorb less minerals than do normal plants. The largest differences involve magnesium, the amounts being 4-5 times larger in normal plants. In early stages of growth normal plants absorb about 4 times more Ca than do flooded ones but the differences are as 2.5 : 1 at later stages. These differences are related causally to the lack of oxygen in the flooded soil and poor development of roots on flooded plants.

REMARQUES SUR LE COMPORTEMENT PHYSIOLOGIQUE DES XEROPHYTES PENDANT LA PERIODE DE DESSECHEMENT DU SOL

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D'une façon générale, la résistance des xérophytes à la sécheresse relève, d'un côté, de caractères structuraux leur permettant de *se protéger contre des déficits excessifs du bilan d'eau,* de l'autre, de propriétés plasmatiques leur conférant *une grande tolérance aux fortes déshydratations ou aux fluctuations rapides dans l'état d'imbibition du plasma.*

Afin d'étudier les réactions des xérophytes pendant la période de dessèchement du sol, des courbes journalières de photosynthèse, de transpiration et de valeur osmotique furent établies chez un certain nombre d'espèces vivaces, non succulentes, xéromorphes et mésomorphes, de la garrigue de la région méditerranéenne française, soit pendant la saison humide au printemps, soit pendant la période de sécheresse estivale.

Les résultats se résument ainsi : 1) Les espèces xéromorphes à feuilles de taille très réduite sont caractérisées pendant l'époque humide par l'absence d'une dépression de l'activité photosynthétique au cours de l'après-midi. 2) Une forte augmentation de la valeur osmotique se produit chez toutes les espèces pendant la saison sèche, même chez celles pourvues de racines atteignant des couches humides. 3) Certaines espèces mésomorphes parviennent à réduire leur transpiration totale journalière pendant la saison sèche jusqu'à la concurrence de 15 % de celle de l'époque humide, soit plus que les espèces xéromorphes étudiées (30 %). 4) Certaines espèces parviennent à réduire leur transpiration au cours de la journée, sans que la photosynthèse en soit influencée. 5) Chez certaines espèces mésomorphes, l'effet sur la transpiration d'une obturation des stomates semble plus grand pendant l'époque sèche que pendant la saison humide. 6) Chez toutes les espèces mésomorphes, l'augmentation journalière de la valeur osmotique est suivie d'une nette dépression dans l'absorption de CO_2. 7) Chez une espèce mésomorphe, la forte augmentation de la valeur osmotique moyenne pendant la saison sèche n'est accompagnée que d'une réduction insignifiante de la photosynthèse totale.

D'une façon générale, les différences morphologiques qui existent entre les espèces étudiées ne se reflètent que d'une façon incertaine dans le comportement physiologique de ces dernières, exception faite des différences de grandeur des feuilles. La réduction foliaire, par une amélioration de l'alimentation en eau des tissus intervasculaires, semble, en effet, déterminer une stabilisation de l'activité photosynthétique au cours de la journée, sans que, toutefois, le taux journalier total de CO_2 absorbé soit augmenté.

L'augmentation souvent considérable de la valeur osmotique chez les plantes à racines profondes pendant l'époque sèche montre que l'accès direct à l'eau phréatique n'évite pas nécessairement à la plante des difficultés d'alimentation en eau. La résistance à la remontée de la sève dans les racines laisse souvent la plante aux prises avec les mêmes dangers de déshydratation que les xérophytes à système radiculaire peu profond.

La faculté très prononcée de certaines plantes mésomorphes de réduire la transpiration totale pendant la saison sèche semble indiquer la présence d'un mécanisme de protection, indépendant de l'obturation des stomates. En effet, d'une part, l'effet de la fermeture des stomates semble bien plus prononcé pendant l'époque sèche que pendant l'époque humide, d'autre part, certaines espèces parviennent à réduire leur transpiration au cours de la journée sans que la photosynthèse en soit influencée.

L'importance des facteurs plasmatiques dans la régularisation de la photosynthèse est illustrée par le fait que, chez une espèce mésomorphe, une augmentation rapide de la valeur osmotique pendant la journée est accompagnée par une nette diminution de l'absorption de CO_2, tandis qu'une augmentation générale de la valeur osmotique pendant la saison sèche n'est suivie que par une réduction insignifiante de la photosynthèse totale. La plante semble donc rétablir pendant l'époque sèche, malgré le fort déficit de saturation en eau, un nouvel équilibre plasmatique, qui permet une activité photosynthétique égale à celle de l'époque humide ; cet équilibre atteint, les réactions aux fluctuations rapides du bilan d'eau resteront les mêmes que pendant l'époque humide.

En conséquence, de nombreuses singularités structurales chez les xérophytes, en particulier la grande extension du système radiculaire ou la réduction foliaire, peuvent avoir une importance considérable pour amortir les fluctuations rapides du bilan d'eau, mais, en général, leur constitution ne les dispense pas d'une adaptation particulière du plasma visant à assurer une grande tolérance à la dessiccation.

TENEUR EN EAU DE *ZILLA MACROPTERA* CULTIVEE A BENI-ABBES (SAHARA) ET A PARIS

par Paul Binet

Laboratoire de Physiologie végétale de la Faculté des Sciences de Paris

Nous avons suivi le développement de *Zilla macroptera* sous le climat désertique saharien de Beni-Abbès. Les plantes ont été cultivées de septembre 1951 à juillet 1952 dans de grands pots enfoncés dans le sol du jardin du Centre de Recherches Sahariennes et ne recevant aucun arrosage artificiel. Des plantes âgées de 2, 5, 7 et 10 mois ont été récoltées. Nous exposerons ici les résultats relatifs à la quantité d'eau contenue dans une plante et à la teneur en eau des tissus construits à Beni-Abbès ; nous comparerons ensuite ces données aux résultats obtenus avec des plantes cultivées à Paris.

Quantité d'eau contenue dans une plante saharienne.

Un pied de *Zilla macroptera* contient :

1,50	grammes d'eau	à	2 mois
60,05	»	»	5 »
53,00	»	»	7 »
86,07	»	»	10 »

Entre 5 et 7 mois une plante perd de l'eau. Ce phénomène se situe entre janvier et mars, c'est-à-dire à un moment où l'humidité de l'air est grande, les précipitations relativement abondantes et les températures basses.

(Hygrométrie relative de l'air : moyenne mensuelle de janvier, 65 % ; de février, 47 %. — Précipitations totales en janvier et février : 29 mm d'eau. — Températures : moyenne mensuelle de janvier, 9° ; de février, 13°. Minima de janvier, 2° ; de février, 5°.)

La diminution de la quantité d'eau contenue dans une plante est donc à mettre en parallèle avec la diminution de la température. Celle-ci freine énergiquement le ravitaillement en eau des plantes malgré l'atténuation sensible de l'ambiance desséchante où vit la plante.

Remarquons que si l'hiver, du fait de ses températures basses, touche le ravitaillement en eau des *Zilla,* les synthèses, elles, ne sont pas très sensiblement perturbées. En effet, si on suit l'évolution de la quantité de matière sèche contenue dans une plante au cours du développement des *Zilla,* on voit que cette quantité augmente assez régulièrement jusqu'à 10 mois, sans subir de heurts saisonniers.

(A 2 mois : 0,24 grammes de matière sèche dans une plante ; à 5 mois : 13,04 grammes ; à 7 mois : 20.99 grammes ; à 10 mois : 40,77 grammes.)

Teneur en eau des plantes sahariennes.

La quantité d'eau contenue dans 100 grammes de matière fraîche est successivement de :

86,04	grammes à	2 mois
82,16	»	5 »
71,63	»	7 »
67,86	»	10 »

Au fur et à mesure que les plantes vieillissent, elles sont de moins en moins riches en eau. Cet appauvrissement en eau est fortement accéléré en hiver. Il n'existe donc aucune corrélation entre la teneur en eau des plantes et l'humidité ambiante (état hygrométrique de l'air et précipitations).

Comparaison avec des Zilla *cultivées à Paris.*

Des *Zilla macroptera* ont été cultivées à Paris en utilisant le même sol et la même méthode qu'à Beni-Abbès. Mais à Paris les plantes se sont développées d'avril à novembre 1952. Les récoltes ont eu lieu à 2, 5 et 7 mois.

La quantité d'eau contenue dans une plante parisienne passe par un maximum à 5 mois (mois d'août). Après ce stade, dès les premiers froids, la plante perd de nombreux rameaux feuillés. Sauf au stade 2 mois, la quantité d'eau fixée par une plante est toujours plus grande à Beni-Abbès qu'à Paris.

De même les tissus végétaux construits au Sahara sont toujours plus riches en eau que ceux formés sous le climat parisien, sauf au stade 2 mois. En effet, à Paris, nous avons successivement 86,45, 75,60 et 70,09 pour cent d'eau dans la matière fraîche des *Zilla*.

Conclusions. — C'est donc sous le climat le plus sec mais aussi le plus chaud que les *Zilla macroptera* fixent le plus d'eau et forment les tissus les plus hydratés, à condition de considérer des plantes suffisamment âgées. Pour celles-ci, en ce qui concerne les échanges d'eau, le facteur limitant n'est pas la sécheresse mais le froid. C'est lui qui ralentit le ravitaillement en eau des *Zilla* désertiques en hiver. Par contre, ce sont les températures élevées de la belle saison saharienne qui permettent aux plantes de Beni-Abbès de construire, malgré la sécheresse, des tissus mieux hydratés que ceux des *Zilla* parisiennes.

WATER SUPPLY AND DROUGHT RESISTANCE AS FACTORS AFFECTING THE COMPOSITION OF BRYOPHYTE POPULATIONS IN ARABLE FIELDS

by E. A. C. L. E. Schelpe
University of Capetown

The ability of some bryophytes, mainly mosses and acrogynous Jungermanniales, to survive long periods of drought is a well-known phenomenon. By contrast, other bryophyte gametophytes are unable to survive even short periods of drought and require a continuous, as opposed to an intermittent, water supply for survival.

The marked effects of differences in the periodicity or continuity of moist conditions on various bryophytes have been noted in populations of small bryophytes growing on cultivated fields near Oxford, England. These bryophyte populations are usually catastrophically destroyed by ploughing

each year. They develop under growing crops, especially cereals, and continue to live among the « stubble » left after such cereals have been reaped. The humidity near the soil surface under crops is usually high and dew is frequently deposited at night. Consequently, the bryophytes in this habitat dry out infrequently. However, in unusually dry summers, such as that which occurred in 1949, the bryophytes may dry out for comparatively long periods.

The effect of summer drought on these bryophyte populations can be illustrated by a comparison of the density and composition of the populations which developed on the same field under wheat in two successive years. The nitrogen contents of the soils were similar in both years.

In the autumn of 1948, this field, and three other adjacent fields on the same soil type, supported a rich growth of hepatics. The following counts of individual plants of each of the hepatic species in a total of ten 1 sq. dm. quadrats were obtained : *Anthoceros punctatus,* 49 ; *Fossombronia wondraczeki,* 336 ; *Riccia glauca,* 12 ; *Riccia sorocarpa,* 24. In the autumn of 1949, it was found that *Anthoceros* and *Fossombronia* were absent from the same four fields and that only three small *Riccia* thalli occurred in ten 1 sq.dm. quadrats.

The acrocarpous mosses forming part of this population showed a marked difference in the quantity of gametophores produced in 1948 and 1949 on this field. e. g. 24325 gametophores in ten 1 sq.dm. quadrats in 1948 ; 3264 in the same area in 1949. However, there was little difference in the number of species involved. Of ten moss species, only *Ephemerum serratum* failed to produce gametophores in 1949.

The difference in the bryophyte populations in 1948 and 1949 can be correlated with moisture conditions prevailing during the summers of these two years. During the summer drought of 1949 only 6.56 ins. of rain fell between April and August inclusively as compared with 13.02 ins. during the same period in 1948. The number of rainy days during these summer periods was 41 days in 1949 in contrast to 73 days in 1948. In 1949, the late rains in October were preceded by a dry period lasting from March until September with drought in June and July. In 1948, moist conditions prevailed throughout the summer except for a mild drought in July.

Consequently, the absence of *Anthoceros* and *Fossombronia* and a reduced number of moss gametophores and *Riccia* thalli per unit area can be correlated with summer drought of long duration.

The validity of the correlation is borne out by the reaction of these bryophytes to drought in culture. Mature plants of *Anthoceros punctatus* and *Fossombronia wondraczeki* have failed to survive four days' complete drought and thalli of *Riccia glauca* and *R. sorocarpa* have died after a week's drought at laboratory temperatures in sand culture. In contrast, gametophores of *Ceratodon purpureus* and *Dicranella varia* as well as other constituent moss species in culture have not been killed by prolonged drought.

However, gametophore production is considerably reduced by intermittent droughts, which damage the protonemata from which further gametophores would be produced.

Although these mosses are able to withstand drought, it is evident that the duration and continuity of water supply affects the quantity of gametophores per unit area. In contrast to the hepatics, the continuity of moist conditions, though favourable, is not essential, except in cases such as *Ephemerum* where the protonema constitutes a large part of the plant body. The quantity and size of hepatic colonies in comparable fields depends largely on the duration of continually moist conditions and the absence of summer drought in the micro-climate.

VERSUCH EINER KONTINUIERLICHEN GASANALYTISCHEN TRANSPIRATIONSREGISTRIERUNG

von Bruno Huber
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Die grossen Fortschritte der Kohlensäureschreibung (Ultrarot-Absorptionsschreiber) lassen es wünschenswert erscheinen, neben den Vorgängen der Assimilation und Respiration auch den dritten wichtigen Gaswechselvorgang, die Transpiration mit einer vergleichbaren gasanalytischen Methode zu registrieren. Der Wasserdampfschreiber « Thermoflux » der Badischen Anilin- und Sodafabrik Ludwigshafen wurde für diesen Zweck geprüft und weiterentwickelt. Er registriert den Wassergehalt eines ständig laufenden Luftstroms thermoelektrisch auf Grund der Lösungswärme in konzentrierter Schwefelsäure. Als gefährlichste Fehlerquelle gilt es Wasserdampfkondensationen in den Leitungswegen vor Erreichung des Messgerätes zu vermeiden.

RECHERCHE D'UN ENREGISTREMENT DE LA TRANSPIRATION PAR ANALYSE DE GAZ CONTINUE

Il serait intéressant de pouvoir enregistrer parallèlement aux échanges de gaz carbonique (respiration et photosynthèse) les pertes de vapeur d'eau de la plante. L'auteur essaye d'utiliser dans ce but l'appareil « Thermoflux ». La teneur en eau de l'air circulant à travers l'appareil est enregistrée grâce au dégagement de chaleur libérée lors de la fixation de l'eau par de l'acide sulfurique concentré.

LIMITING FACTORS OF TRANSPIRATION

by F. van der Paauw
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The daily course of transpiration was determined by means of 1, 1/2 hourly weighings. Large fluctuations were observed (example in fig. 1). The behaviour of some plant species is very different. Obviously tops and depressions are not directly related to the external conditions. It seems likely that these fluctuations are mainly due to stomatal activity.

The effect of temperature on the daily transpiration of oats is very

distinct. High humidity of the air is only limiting in the case of low temperature. Further a moderate effect of cloudiness and no influence of wind were found.

With willows the degree of cloudiness is dominant. The influence of temperature is less. The impression is that higher temperatures do not enhance transpiration at all. The influence of air humidity proved to be almost negligible. There is no influence of wind.

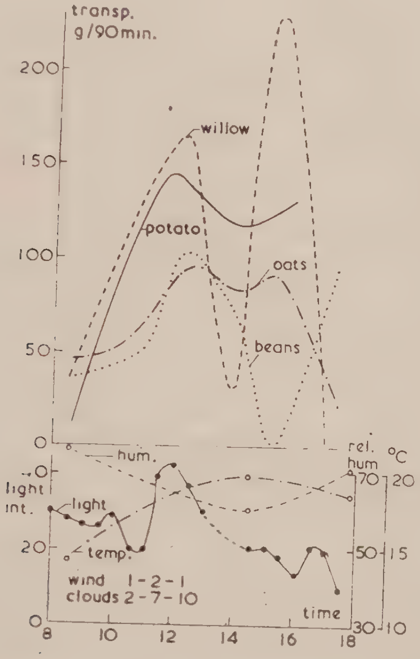

Fig. 1. — Course of transpiration of some plants at 21-7-1949 and meteorological factors (light in arbitrary units, wind and clouds at 8, 14 and 19 hrs. resp.).

Probably these results have also been affected by stomatal action. By applying a similar analysis to those 1 1/2 hour periods in which the daily maximum rate of transpiration occurred, this factor may be eliminated to a large extent. It may be assumed that the stomates are rather wide open at that time. A comparison with the results obtained before will give some information on the effect of stomatal activity.

Again a strong influence of temperature on the rate of maximum transpiration of oats is noted (fig. 2). The influence of air humidity is slight. No influence of cloudiness is found (fig. 3 a), possibly with the exception of heavy cloudiness (this coincides, however, with high humidity of the air, so that the real cause of the slight depression is uncertain). There is no influence of wind (fig. 3 b).

With willow the effect of temperature is more marked than before, the influence of cloudiness, however, is less pronounced. The influence of humidity is slight. Again no influence of wind was found. It is interesting that the effect of temperature has been found to be considerably larger.

It may be concluded that the large effect of temperature has been moderated during the rest of the day, probably by means of stomatal adaptation. It follows from the decrease of the influence of cloudiness that this factor is of minor importance if the stomates are rather wide open and no stomatal regulations interfere. Perhaps with willow (with its probably quickly reacting stomates) a period of 90 minutes is still too long for a complete elimination of this factor.

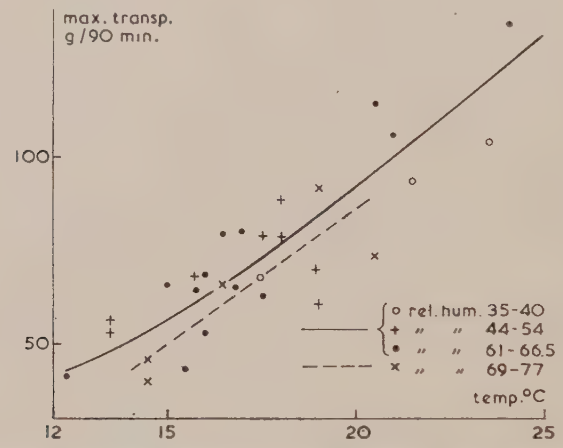

Fig. 2. — Relation between temperature and rate of daily maximum transpiration of oats at different relative humidity (no corrections).

In contradiction to the marked effect to temperature the significance of other physical factors, normally influencing the evaporation of water, is remarkably small. This result seems to be contradictory to the conception of a strictly stomatal control.

On the other hand the close relation found between temperature and transpiration suggests a direct causal relationship. The values of Q_{10} being very high, it is unlikely that the increase of the rate of transpiration with rising temperature is merely due to an increasing vapour pressure. The permeability to water of the protoplasm (conductivity) might therefore be seen as a probable limiting factor of transpiration. The high values of Q_{10} found in the case of water permeability (De Haan) support this supposition. It has, however, been observed that marked variations of soil temperature do not considerably affect transpiration so that a possible influence of water permeability must be restricted to the protoplasm of the leaf cells.

Limitation of the rate of transpiration by stomatal control might occur (1) if the losses of water are very much in excess of the absorption (mid-day depressions), (2) in the case of decreasing light intensity (especially with willow), (3) by excess of water in the morning and deficiency in the afternoon, leading to late opening and early closing (at days of a strong transpiration on an average 80 % of the amount transpired in 24 hours was consumed between 8 a.m. and 5 p.m. both with oats and willow, though the length of the daytime was about 16 hours).

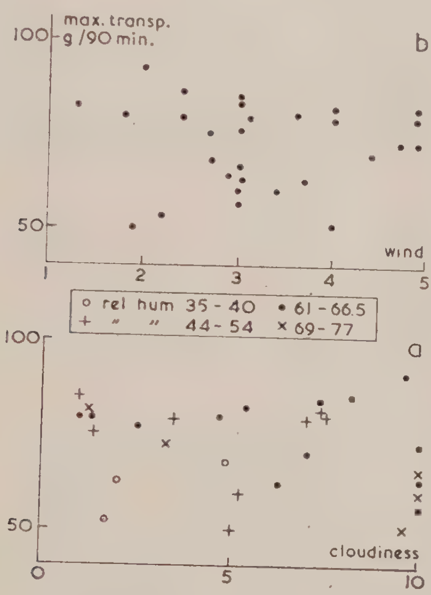

Fig. 3. — Relation between degree of cloudiness (a), wind velocity (in degrees Beaufort) (b) at different rel. humidity (for a. only) and rate of maximum transpiration of oats, after correction for the influence of temperature. The influences of cloudiness-temperature (a) and wind-temperature correlationships (b) have been eliminated.

Physical factors, such as humidity and wind, are not limiting under normal conditions. Only if the humidity is very high, the limiting effect of this factor is found. In this case probably the stomates are also less open.

It might be possible that the importance of these factors is more pronounced in the case of a close growing crop. Pots with oats placed amidst the field-crop showed indeed a marked depression of transpiration. However, numerous observations have proved that the course of transpiration in this case is almost identical with that of pots outside the crop, though

at a lower level. Probably this would not be so if the transpiration of the whole plants had been determined by deviating microclimatological conditions. This might be explained by the fact that top leaves and panicles probably more or less behave like detached plants, whereas the transpiration of lower leaves and stems is strictly limited by high humidity and closure of the stomates. The course of transpiration is, however, unchanged and its rate apparently depends to a large extent on the same factors as that of separate plants.

LA TRANSPIRATION VEGETALE ET LE COMPLEXE CHALEUR-EVAPORATION DANS LA FRANCE MEDITERRANEENNE

par M. Godard

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On envisagera dans cette note l'influence de la température de l'air qui, avec le rayonnement solaire et le pouvoir évaporant de l'air, interviennent d'une manière prépondérante dans les phénomènes transpiratoires de la plante.

Les essais entrepris à la Station de Bioclimatologie agricole de Montpellier (Institut National de la Recherche Agronomique) ont porté sur le Maïs (variété Maïs de Tarbes) cultivé en vases de végétation. Du point de vue biologique, des pesées journalières à partir de la floraison mâle des plantes faisaient connaître la quantité d'eau transpirée entre 6 et 17 h. TMG qui représente la majeure partie de l'eau émise au cours du nycthémère. Grâce à des déterminations régulières de surface foliaire, on pouvait en déduire la quantité d'eau perdue par décimètre carré de surface ou *taux de transpiration moyen diurne.* Enfin, l'humidité des vases était maintenue constante par adjonction fréquente au cours de la journée.

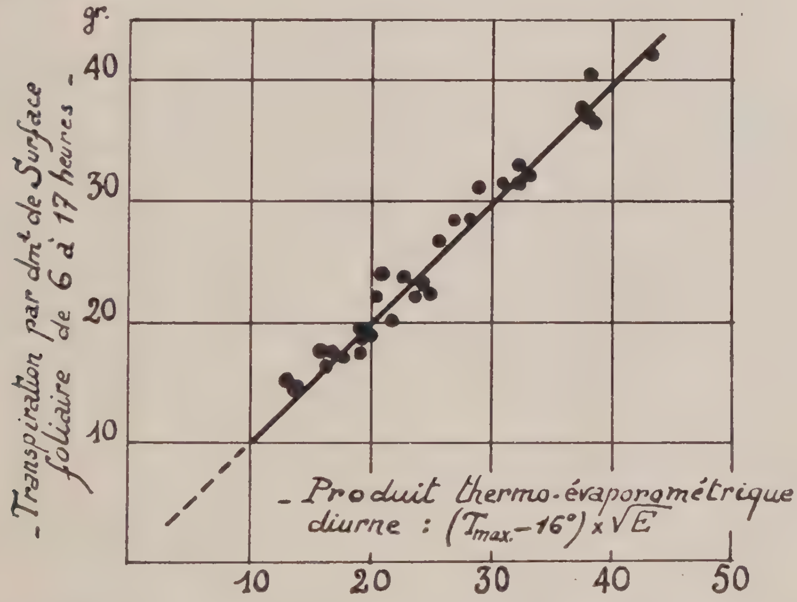
Du point de vue physique, on a pris comme test de la *température moyenne diurne* de l'air, *la température maximum* relevée sous l'abri météorologique classique. Le *pouvoir évaporant* de l'air était lui-même défini par les mesures faites entre 6 et 17 h. à l'évaporomètre de Piche, également sous abri. Enfin, l'intensité du rayonnement solaire (éclairement) était caractérisée par la *radiation globale* (quantité d'énergie d'origine solaire en cal-gr/cm^2 reçue sur une surface horizontale).

Dépouillement des observations. — Pour tenir compte de l'influence du rayonnement solaire, on a retenu parmi les journées d'observations seulement celles marquées par un *temps clair à peu nuageux.* A l'époque des essais, en juillet-août, le rayonnement global reçu entre 6 h. et 17 h. au cours de ces journées présente une valeur sensiblement identique. Dans ces *condi-*

tions de rayonnement constant, les variations de l'intensité transpiratoire d'une journée à l'autre sont donc à rapporter uniquement à l'action propre de la température et du pouvoir évaporant de l'air.

Un dépouillement adéquat a tout d'abord montré qu'à *rayonnement global et à pouvoir évaporant de l'air constants,* la transpiration, faible lorsque la température maximum ne dépasse pas 16°C (*), augmente linéairement avec le maximum thermique diurne jusqu'au voisinage de 36°C, température la plus élevée observée au cours des essais. D'une manière très approchée, le taux de transpiration diurne apparaît ainsi proportionnel à l'expression $(T_{Max} -16°)$.

Enfin, à *rayonnement global et à température de l'air constants,* l'intensité transpiratoire apparaît proportionnelle à la racine carrée du pouvoir évaporant de l'air. L'augmentation de la transpiration due à un même accroissement du pouvoir évaporant est d'autant plus faible que ce dernier est lui-même plus élevé.



Il résulte de ces faits que par journée claire, la transpiration des plantes doit dépendre du *produit thermo-évaporométrique* $(T_{Max}-16°) \times \sqrt{E}$ où T_{Max} est la température maximum de l'air et E le pouvoir évaporant de l'air. C'est ce que fait ressortir le graphique ci-dessous résumant les observations relatives à l'une des années d'études, en juillet-août 1948, à partir

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(*) Un tel maximum diurne correspond à une température moyenne nycthémérale de l'ordre de 10° qui est précisément voisine du zéro apparent de végétation du Maïs.

de la floraison mâle des plantes. Au cours de cette période, les maxima thermiques journaliers s'échelonnèrent de 23° à 36°C et les évaporations diurnes varièrent de 1,5 mm à 5,5 mm. Pour chaque journée, on a porté en abscisse le produit thermo-évaporométrique et en ordonnée le taux de transpiration correspondant.

Pour l'ensemble des journées, le graphique montre qu'il existe une corrélation étroite entre l'intensité transpiratoire et le produit thermo-évaporométrique. Par temps clair, cette fonction climatique apparaît donc susceptible de caractériser l'action du *complexe chaleur-évaporation* sur la transpiration.

En raison de la grande luminosité de l'été méditerranéen dont la fraction d'insolation s'élève à 0,71 à Montpellier, la considération du produit thermo-évaporométrique peut permettre, en outre, de rendre compte des pertes d'eau de la plante *in situ*. C'est ce que nous avons pu observer dans les conditions du plein champ en 1952, où l'été fut particulièrement sec et ensoleillé, sur une culture de Maïs irrigué parvenue à son plein développement. La variation du taux d'humidité dans la couche de 10 à 30 cm de profondeur où la plante puise avec intensité s'est montrée en rapport direct avec le produit défini ci-dessus. La différence d'humidité contenue dans cette couche observée d'une part après une irrigation et d'autre part avant l'irrigation suivante a été en corrélation étroite avec les sommes des produits thermo-évaporométriques journaliers afférents à la période considérée.

Ainsi, dans le Sud de la France où les périodes ensoleillées sont souvent longues, la considération journalière des produits thermo-évaporométriques obtenus à partir de *données climatiques simples* devrait permettre d'évaluer l'humidité du sol et de prévoir par conséquent la date des irrigations.

Référence : M. GODARD, *C. R. Ac. Sc.*, CCXXIX, 1949, pp. 850 et 900.

2. *MECANISME DU GEL ET RESISTANCE AU FROID VERNALISATION*

Mécanisme du gel et résistance au froid

RAPPORT

LE MECANISME DU GEL ET LA RESISTANCE AU FROID

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Le présent travail étant une mise au point, nous nous sommes limité, dans notre bibliographie, aux publications récentes, excepté lorsque l'exposé du sujet demandait un certain recul. — Nous avons traité le problème au point de vue biophysique, ce qui a occasionné parfois l'inclusion de travaux de physique pure. — Le sujet assigné nous a paru double ; nous l'avons donc divisé en deux parties.

I. LE MECANISME DU GEL

Nous avons publié en 1940 une revue assez détaillée de ce problème (Luyet et Gehenio). Cette date sera donc notre point de départ. — La congélation et les phénomènes connexes seront analysés sous quatre chefs principaux : 1. Formation des noyaux cristallins ; 2. Croissance des cristaux et propagation de la glace ; 3. Solidification à l'état amorphe ; 4. Questions diverses.

Formation des noyaux cristallins. La bibliographie des recherches biologiques sur ce sujet est très pauvre, elle se réduit à quelques observations faites au hasard dans la nature et à quelques rares études expérimentales. Mais, tandis que les biologistes ont montré peu d'enthousiasme pour ces recherches, les physiciens les ont poursuivies avec vigueur et sont arrivés, ces dernières années, à des résultats qui ont révolutionné nos concepts sur plusieurs points fondamentaux.

La cristallisation spontanée de l'eau surfondue a été l'objet d'une étude détaillée de M. Dorsey, du *Bureau of Standards* à Washington. Ce savant a montré, entre autres, que : (1) La température de cristallisation d'un échantillon donné d'eau demeure constante, à environ un degré près, dans des expériences répétées ; par exemple, elle n'a varié que de —17°0 à —17°8 dans 17 expériences faites dans l'espace de 2 jours. (2) Cette température varie d'un échantillon à l'autre, étant, par exemple : —6°8, —10°5 et

—16°0, respectivement, dans 3 échantillons différents. (3) Au-dessus de la température à laquelle un échantillon cristallise, l'agitation est généralement sans effet. (4) Il n'est pas très rare de trouver des échantillons qui restent en surfusion pendant longtemps à —20° ou au-dessous.

Le problème général de la formation des noyaux cristallins dans un liquide surfondu a été étudié, entre autres, par les métallurgistes et les météorologistes (par ces derniers, à cause de son importance pour la précipitation des nuages). Un certain nombre de physiciens que ces sujets intéressent ont organisé des colloques périodiques et publient (en forme miméographiée) les comptes rendus de leurs séances. Leurs travaux ont abouti aux conclusions fondamentales suivantes : (1) Les liquides en surfusion peuvent être classés en deux catégories : ceux, très nombreux, qui cristallisent généralement sous l'action de particules inoculatrices étrangères et ceux, plutôt rares, qui forment leurs propres noyaux. (2) Les particules inoculatrices étrangères produisent la cristallisation à des températures déterminées dépendant de leur nature particulière. (3) Les liquides qui forment leurs propres noyaux fournissent des courbes température-temps explicables par la théorie des rencontres au hasard des molécules formatrices (voir, par exemple, TURNBULL et VONNEGUT, 1952).

Comme l'eau est dans la catégorie des substances qui cristallisent généralement sous l'action de particules étrangères, le mécanisme de la formation des noyaux et les particularités de la surfusion dans les tissus vivants doivent être réétudiés. Comme, d'autre part, l'ensemencement cristallin entre cellules voisines ne se fait pas librement, ainsi que nous allons le voir, il est possible que la surfusion joue un rôle beaucoup plus considérable qu'on le croit généralement dans la survie des tissus au gel, en particulier dans les régions arctiques.

Nous avons montré en 1937 (LUYET et GIBBS) que les membranes cellulaires empêchent ou retardent l'ensemencement cristallin d'une cellule gelée à sa voisine en surfusion (ce phénomène sera démontré dans un film cinématographique). — BERGH (1948) a présenté quelques observations nouvelles sur la résistance des membranes cellulaires au passage de la glace. — Notre élève GALOS (1941) a remarqué que les membranes artificielles de cellulose offrent également un obstacle à la propagation de la glace. — LUSENA et COOK (1953), du *National Research Laboratory*, à Ottawa, dans une étude systématique de ce problème, ont montré que les membranes perméables à l'eau ne sont souvent pas perméables à la glace.

La découverte de la propriété de particules étrangères déterminées, de faire cristalliser l'eau à des températures fixes (cf. V. SCHAEFER, 1953), a suggéré l'introduction de substances inoculatrices diverses dans les fluides de l'organisme et dans les solutions ou suspensions aqueuses en usage dans les laboratoires de biologie. LUSENA et COOK (travail sous presse) ont employé l'iodure d'argent hexagonal et le carbonate de calcium préalablement traité à haute température. Nous employons couramment le béryl naturel.

CROISSANCE DES CRISTAUX ET PROPAGATION DE LA GLACE. MERYMAN, du *Naval Medical Research Institute,* à Washington, a montré que les cristaux de glace croissent à —130° et qu'à —80° ils passent de 200 à 10 000 angströms (où ils sont visibles au microscope) en 8 minutes. Il avait d'abord produit de la glace amorphe en condensant, dans le vide, de la vapeur d'eau sur une plaque métallique maintenue à —195°. Ayant alors élevé la température de cette glace amorphe, il a assisté à sa transformation en cristaux, dont il a photographié les moulages métalliques au microscope électronique. Les cristaux avaient crû aux dépens de la masse amorphe et ensuite aux dépens des cristaux plus petits qui s'étaient sublimés. (Ce travail est sous presse. Nous projetterons les photographies que M. MERYMAN a bien voulu nous prêter.)

Dans un travail actuellement en cours dans notre laboratoire, nous étudions une deuxième étape de la croissance des cristaux de glace : la croissance à partir des dimensions observables au microscope à celles observables à l'œil nu. Les résultats préliminaires indiquent une accélération énorme de la croissance aux abords immédiats du point de fusion et des variations considérables avec la nature et la concentration de substances en solution.

La question déjà ancienne de la dimension des masses de glace qui se forment, à différentes vitesses de refroidissement, dans les gels ou les tissus, a été reprise avec l'outillage et les méthodes modernes par MERYMAN (1953).

La vitesse de propagation de la glace dans les solutions et gels, en fonction de la température, de la vitesse de refroidissement, de la composition et concentration du milieu, dans des systèmes de formes et de dimensions données, a aussi été étudiée dans les articles que nous avons mentionnés de MERYMAN (1953) et de LUSENA et COOK (I, 1953 ; II, sous presse). Nous avons nous-même un travail en cours sur ce sujet ; quelques observations ont été enregistrées sur film et seront projetées.

On sait depuis longtemps que plusieurs organismes, végétaux et animaux, survivent à la formation de glace dans leurs tissus. La question qui se pose à leur sujet est celle de la quantité de glace qu'ils peuvent supporter. Mais cette question n'a eu que peu d'attrait pour les physiologistes contemporains. Nous ne connaissons qu'un groupe d'auteurs qui aient tenté récemment des déterminations de la quantité de glace présente dans les plantes à différentes températures : ce sont SCHOLANDER, FLAGG, HOCK et IRVING (1953) dans une étude sur des lichens. Malheureusement, ils n'ont pas considéré la question de la relation entre le degré de congélation de ces plantes et leur survie.

SOLIDIFICATION A L'ÉTAT AMORPHE. En 1937, nous avons introduit en biologie l'idée de la solidification à l'état amorphe par la méthode du refroidissement rapide (vitrification). Les prévisions que cette méthode permettait de faire sur la survie ont été abondamment confirmées par les résultats obtenus (survie de cellules, tissus et organismes, végétaux et animaux, reprise des battements de cœur et reprise du développement de l'embryon de poulet, etc. ; cf. notre récente revue : LUYET, 1951). Mais l'étude de l'aspect physique du phénomène n'a pas avancé de pair avec les applications biologiques.

Pour reconnaître l'état vitreux, les physiciens emploient généralement l'analyse aux rayons X ou au microscope électronique, et l'examen au microscope polarisant. Les deux premières méthodes permettent de déceler des noyaux cristallins de l'ordre de quelques dizaines d'angströms, mais le microscope polarisant ne révèle que des cristaux plusieurs centaines de fois plus gros, de la dimension de la longueur d'onde de la lumière visible (environ 0.5 micron). On peut, plus simplement, se servir de l'éclairement latéral sur fond noir, qui met en évidence des particules de quelques milliers d'angströms ; mais, dans ce cas, on doit savoir à l'avance que les particules observées sont des cristaux. — On doit donc remarquer que, lorsqu'on parle de structure amorphe ou vitreuse, on veut dire une structure dans laquelle on n'a pas observé de cristaux mesurant plus de quelques dizaines ou de quelques milliers d'augströms, selon la méthode employée.

Il a été démontré par l'analyse aux rayons X que l'eau pure (BURTON et OLIVER, 1935) et les solutions de gélatine (BARNES et MATTHEWS, 1939) peuvent être obtenues à l'état vitreux (au sens défini) par refroidissement rapide. Mais, pour le protoplasme vivant lui-même, nous n'avons encore que des observations au microscope polarisant ou à l'ultra-microscope, et, encore, ces observations nous renseignent davantage sur l'état physique du suc cellulaire que sur celui de la substance protoplasmique elle-même. Dans un travail actuellement en cours dans notre laboratoire, nous étudions, à l'aide du microscope électronique, la croissance des cristaux dans des gels et du protoplasme traités par refroidissement rapide et réchauffés dans des conditions diverses.

Nous ne savons à peu près rien des propriétés physiques des solutions aqueuses à l'état vitreux, telles que la densité, la chaleur spécifique, la chaleur de fusion, la conductibilité calorifique et électrique, la pression de vapeur, la constante diélectrique, etc. Il y a déjà plusieurs années que nous avons inscrit l'étude de ces propriétés à notre programme de recherches, mais elle attend toujours son tour. Nos connaissances sur la température à laquelle a lieu le passage de l'état vitreux à l'état cristallin (dévitrification) se limitent à une demi-douzaine de valeurs (LUYET, 1939 ; LUYET et GOMEZ, 1947). Nous ne savons également que peu de chose sur ce qui se passe réellement aux changements d'état : dévitrification, vitrofusion, vitrosublimation, en particulier, sur la phase ou les phases de la solution qui, actuellement, changent d'état.

QUESTIONS DIVERSES. *Solidification eutectique.* D'après la théorie classique, lorsque la température d'une solution aqueuse est abaissée graduellement, l'eau seule se solidifie pendant un certain temps et la solution devient de plus en plus concentrée. Mais, à une concentration et à une température données, appelées eutectiques, l'eau et la substance dissoute se précipitent, à la fois, à l'état solide. Y a-t-il une solidification eutectique pour le suc cellulaire ? Cette question, qui a déjà été vivement discutée en physiologie végétale, il y a cinquante ans, à la suite des travaux de MEZ (1905) et VOIGTLAENDER (1909), est encore à l'ordre du jour. Mais elle est peut-être mal posée. Au lieu de se demander s'il y a un changement particulier de structure qui mérite le nom d'eutectique, ne doit-on pas plutôt se demander quels sont, de fait, les changements de structure décelables à différentes températures, sans préjuger de leur nature ? Le problème se ramène alors à celui des « températures critiques » que nous allons maintenant envisager.

Températures critiques. On rencontre, de temps à autre, en physiologie végétale, la notion de températures critiques auxquelles les plantes ou tissus végétaux sont particulièrement susceptibles. JOSLYN et DIEHL, par exemple (1952), mentionnent des cas où « le contenu des cellules était dans un état de désintégration plus accentué » après que les tissus avaient été soumis aux températures de —12° à —18°. BERRY et coll., et aussi BEDFORD ont également relevé une action léthale plus intense sur les bactéries à ces mêmes températures (cf. JOSLYN et DIEHL, *l. c.*). En physiologie animale, nous connaissons plusieurs cas de prétendues températures critiques entre —15° et —80°. Le sujet demande une étude systématique.

Effets de la rapidité du refroidissement et du réchauffement. Plusieurs travaux ont été publiés sur ce sujet, quelques-uns en physiologie végétale et un plus grand nombre en physiologie animale. (Le lecteur trouvera les premiers cités dans la revue de LEVITT, 1951.) Mais, en général, les auteurs, après avoir donné les résultats d'observations ou d'expériences isolées sur des sujets variés, ont suivi la coutume de se ranger parmi les partisans de l'une ou l'autre des quatre théories d'après lesquelles c'est : (1) le refroidissement lent, (2) le refroidissement rapide, (3) le réchauffement lent, (4) le réchauffement rapide, qui cause le plus de dégât. Les résultats sont chaotiques. On ne s'entend pas sur ce qu'on appelle rapide ou lent, on ne tient pas compte des différences de contenus en eau des tissus étudiés, de leurs différences de structure, de dimensions, de conductibilité, des différences de quantités de glace formée, des différences de températures actuelles de cristallisation, etc. Le besoin d'une étude systématique organisée se fait vivement sentir dans ce domaine.

Constantes physiques. Les constantes physiques du matériel étudié, telles que la chaleur spécifique, la chaleur de fusion, la conductibilité, pour différents contenus en eau ou en glace, à différentes températures, n'ont été déterminées que pour un petit nombre de tissus végétaux. Parmi les travaux récents sur ce sujet nous mentionnerons ceux sortis des laboratoires de l'école d'ingénieurs de l'Université du Texas et portant les noms de WOOLRICH, STAPH, SHORT et BARTLETT (pour références, voir STAPH et WOOLRICH, 1951).

Questions de technique. Parmi les techniques nouvelles, nous mentionnerons les suivantes : (1) MERYMAN (1953) a décrit un appareil, basé sur le principe de la réflexion des ondes électromagnétiques, pour déceler des masses de glace à l'intérieur des tissus et mesurer leurs dimensions. (2) SCHOLANDER et coll. (1953) ont déterminé la quantité de glace présente dans des objets très petits en mesurant leur densité par la méthode de flottement. (3) LUYET et GONZALES (1951) ont enregistré, au moyen d'un oscillateur à rayons cathodiques, des vitesses de refroidissement de l'ordre de 150.000 degrés par seconde. — Nous mentionnerons également ici l'usage, en physiologie végétale, de la méthode relativement récente de dessiccation après congélation. SVIHLA et OSTERMAN (1943) ont obtenu la survie de graines d'orchidées ainsi traitées.

II. LA RESISTANCE AU FROID

Les travaux récents sur la résistance des plantes au froid ont été revus et analysés, en 1951, par notre ami M. LEVITT, de l'Université du Missouri. Nous nous bornerons donc ici, après un rappel des points principaux discutés par lui, à quelques brèves additions et à la discussion de trois questions qui ont été ravivées récemment : la synérèse, la déshydratation naturelle et l'action de solutions protectrices.

SUJETS PRÉCÉDEMMENT REVUS. La revue de M. LEVITT discute : (1) la valeur comparative des méthodes de contrôle de l'endurcissement au froid en plein air et au laboratoire, (2) l'effet de la vitesse de refroidissement sur les dégâts causés par le gel, (3) l'activité respiratoire et assimilatrice au-dessous de 0°, (4) les facteurs externes de l'endurcissement au froid, tels que la fumure ou l'exposition à la lumière, et les changements internes qui accompagnent l'endurcissement, comme l'accumulation de sucres et d'acides aminés, la liaison colloïdale ou osmotique en général, l'augmentation de la proportion de protéines solubles (mise en évidence par SIMINOVITCH et BRIGGS de l'école de Montréal) et une prétendue formation de substances lipoïdiques.

INFORMATIONS COMPLÉMENTAIRES. *Phénomènes divers.* Parmi les changements d'ordre physique ou chimique capables d'influencer l'endurcissement au froid nous ajouterons les suivants à la liste qui vient d'être donnée (quoique leurs auteurs ne les aient pas toujours présentés comme ayant affaire avec l'endurcissement). Ces changements sont initiés ou causés par le froid lui-même, avec ou sans congélation. — NORD a étudié, depuis plus de 25 ans, la désagrégation et, dans certains cas, l'agrégation des particules colloïdales, produites par la congélation. Dernièrement, il a examiné les conditions dans lesquelles il y a agrégation ou désagrégation (cf. LEICHTER, 1951). — ULLRICH (1941) a décrit un réarrangement moléculaire résultant en la formation de filaments biréfringents dans des mélanges de gélatine et d'agar gelés à —10°. — W. H. SMITH (1951) a attiré l'attention des chercheurs sur la rupture de l'équilibre des échanges métaboliques produits par le simple abaissement de température, sans congélation. — GENEVES (1951) a rapporté des observations cytologiques qui sembleraient indiquer que l'affinité des protéines pour l'eau diminue avec la température, en l'absence de congélation. — Les changements nombreux résultant de l'activité des enzymes dans les tissus végétaux gelés ont été revus en détail par JOSLYN (1949). — KIERMEIER (1951) a considéré spécialement l'altération des enzymes elles-mêmes par le gel et les modifications de l'activité enzymatique qui s'ensuivent. — SCHOLANDER et coll. (1953) ont mesuré des échanges respiratoires dans les plantes gelées, maintenues à —26°, et ont observé qu'au moment de la congélation le coefficient Q_{10} de la respiration devenait subitement dix fois plus grand.

Synérèse. La synérèse — une exsudation d'eau d'un gel, résultant d'un réarrangement moléculaire — a été invoquée par plusieurs auteurs pour expliquer certains phénomènes physiologiques (GICKLHORN et MOESCHL, 1930 ; WEBER, 1935). Nous l'avons nous-même proposée (GEHENIO et LUYET, 1939) pour expliquer *la mort* par le froid, *en l'absence de glace,* dans le plasmode des myxomycètes dont on peut voir le protoplasme se séparer, à la suite d'un abaissement de température, en une phase aqueuse et un coagulum. D'autre part, BECQUEREL, qui avait signalé, en 1937, une contraction du cytoplasme et des noyaux des épidermes végétaux *au moment du gel,* avait aussi considéré ce phénomène comme une synérèse. Il avait interprété la contraction comme résultant d'une séparation des molécules d'eau qui, pour former la glace, quittaient le réseau protoplasmique et ainsi en réduisaient les dimensions. Au moment de la fonte de la glace, le protoplasme pouvait, d'après lui, réabsorber son eau et survivre. Dans une communication récente à l'Académie des Sciences (1953), le vétéran bien connu des recherches sur le froid réaffirme sa position. Mais, comme il l'a remarqué lui-même plus d'une fois, sa théorie n'a pas été comprise. Nous croyons que la difficulté principale que ses collègues ont éprouvée a été celle de faire entrer dans le cadre de la définition de la synérèse une déshydratation du protoplasme *résultant de la cristallisation de l'eau.* Mais nous aurons le privilège d'apprendre les détails de ce phénomène de la bouche même de l'auteur.

Déshydratation naturelle. Le fait que les graines en formation subissent une perte d'eau, en même temps qu'elle deviennent résistantes au froid, pose la question (1) de la relation entre la déshydratation et la résistance et (2) de la possibilité d'une déshydratation naturelle produite par le froid et augmentant le degré d'endurcissement. — SCOTT et CULLINAN (1946) ont montré qu'une déshydratation peut souvent augmenter la résistance au froid. — D'autre part, ULLRICH (1943) a observé que certaines plantes, comme l'ortie, peuvent être gelées solides sans dessiccation préalable. — SCHOLANDER et coll. (1953) mesurant le contenu en eau de plantes gelées, déracinées en février-mars dans l'Alaska, trouvèrent qu'il était tombé spontanément, dans certaines plantes aux trois-quarts, dans d'autres à près de la moitié et, dans quelques-unes, au tiers ou au quart de la valeur qu'il avait au mois d'août. Il est donc possible qu'une déshydratation naturelle joue un rôle important dans la résistance des plantes aux températures extrêmes de l'Arctique (température de —20°, maintenue pendant des mois à 25 cm. dans le sol, d'après les mesures des auteurs). Cependant, la déshydratation n'atteint évidemment pas le point où la plante est immunisée contre l'action de toute température, si basse soit-elle. En effet, les mêmes observateurs ont trouvé que des branches d'arbres ou d'arbustes qui, avec leurs bourgeons, supportent des températures atmosphériques occasionnelles de —60°, sont tuées par immersion dans l'oxygène liquide à —183°.

Solutions protectrices. La méthode d'immersion des objets à faire geler dans une solution dite protectrice a été employée fréquemment dans l'étude de la survie en physiologie animale. Les substances protectrices le plus souvent utilisées ont été le glycol et la glycérine. Notre élève KEANE (1953) a trouvé des propriétés protectrices similaires dans l'acétamide et l'urée. L'usage de ces substances a à peine été tenté chez les plantes. Nous avons cependant réussi à obtenir la survie de quelques embryons de pois cultivés sur milieu nutritif synthétique après traitement dans la glycérine et congélation dans l'azote liquide (LUYET et SUN, en préparation). Les solutions protectrices rendent l'eau des tissus qu'elles pénètrent « ingelable ». Un morceau de tissu, comme par exemple un disque mince de tubercule de pomme de terre, gèlera ou ne gèlera pas, à une température et une vitesse de refroidissement données, suivant la quantité de substance protectrice qu'il a absorbée. Malheureusement, avec la plupart des tissus végétaux essayés, la quantité de substance protectrice nécessaire pour prévenir le gel tue le tissu. Dans le cas de plantules entières, les racines sont tuées avant qu'une quantité appréciable ait pénétré les tiges (LUYET, en préparation).

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FROST DAMAGE TO PLANT TISSUES, INCLUDING A STUDY BY CINEMICROGRAPHY

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Various aspects of the problem of frost damage to plants have been under study by a small team of workers at East Malling Research Station, for some years (Rogers, 1954).

A study of the freezing of plant cells under the microscope, with a cinematographic record of the changes taking place, has given a new insight into the fundamental processes of frost damage to plant tissues.

Suitable plant tissues with large cells were mounted in a drop of water on a special microscope slide, fitted with a thermocouple for temperature recording. Controlled cooling was obtained by placing solid CO_2 on the slide. The freezing temperature varied according to the experiment, from about —1° to —8°C.

A film, showing the freezing of three different types of tissue, has been made. The first part shows a study made on chains of single cells (staminal hairs of *Tradescantia*). Ice crystals are seen growing by accretion while invading the medium round the cells. In most cases the wound where the hair was detached from the stamen provided the channel of ice inoculation to the cells. Following this, inoculation by ice crystals from cell to cell occurred. The process of intracellular freezing, causing coagulation and rupture of the protoplasm and death of the cells was shown.

Sometimes two hairs were cooled together, and inoculation through wounds was prevented in one of them. In the latter case the cells remained supercooled, at temperatures at which cells of the adjacent hair were freezing. The supercooled cells remained undamaged, so confirming that it is intracellular ice formation and not low temperature which is responsible for the frost damage.

In a plasmolysed hair the processes of intra and extra vacuolar ice formation are shown, and the withdrawal of water by freezing cells from adjoining, not yet frozen, ones is clearly visible.

The second part of the film shows the freezing of more complex tissues, namely fruit skin. The freezing shows a scattered pattern due to ice inoculation through the cells below the epidermis which were damaged by stripping the skin from the fruit. Damaged cells become inoculated much more readily than undamaged ones.

The third part shows freezing of a moss leaf (*Pterygophyllum lucens*), which consists of a single layer of cells without intercellular spaces. Free-

zing of the cell walls, followed by intracellular freezing of individual cells, can be seen. The interaction of the depth of supercooling, the rate of freezing and the mode of ice formation is demonstrated. The film ends with the process of longitudinal splitting of cell walls by ice. This formation of ice *between* cells is similar to the intercellular freezing which is common in some types of natural frost damage e. g. the lifting of the skin in leaves and flowers. Such intercellular freezing may not cause serious damage ; indeed, in some cases it may actually protect the adjoining cells by partly dehydrating them.

A fuller description of this work is in preparation.

The help of Mr. N. Macqueen in making the film is gratefully acknowledged.

Reference : Rogers (W. S.), 1954. Some aspects of spring frost damage to fruit and its control. *J. Roy. Hort. Soc.*, **79**, 29-39.

LA CRYOSYNERESE CYTONUCLEOPLASMIQUE JUSQU'AUX CONFINS DU ZERO ABSOLU SON ROLE POUR LA VEGETATION POLAIRE ET LA CONSERVATION DE LA VIE

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C'est un phénomène colloïdal complexe que nous avons trouvé en 1937 dans les cellules végétales au moment de leur congélation. Leur état colloïdal cytonucléo-plasmique se partage en deux phases, l'une où leurs particules organiques, sous l'action du froid, se séparent des molécules d'eau pour se solidifier (ou se vitrifier, d'après M. Luyet), et l'autre dans laquelle les molécules d'eau exsudées vont se cristalliser à part, sous forme de cristallites invisibles. Au dégel, la cryosynérèse sera irréversible ou réversible ; irréversible, si les particules cytonucléoplasmiques synérésées dégelées coagulent entre elles : alors c'est la mort ; réversible, si les particules dégelées reprennent leurs liaisons avec les molécules d'eau liquides pour reformer l'état colloïdal initial : alors, c'est la vie. Pour mettre en évidence la cryosynérèse, il faut mesurer la même cellule, son cytoplasma et son noyau, avant, pendant et après l'action du froid, en l'observant au microscope dans le cryostat ou en dehors, quelques secondes avant le dégel. La diminution de volume indique la grandeur de la cryosynérèse cellulaire. *C'est donc un phénomène mesurable bien distinct de ceux de la plasmolyse de la coagulation et de la solidification, quel qu'en soit le mode.* Dans le cas de la cryosynérèse irréversible des cellules vivantes gonflées d'eau (60 à 100 %), à n'importe quelle basse température, au-dessous du point de surfusion, on voit, dès que le dégel est terminé, la coagulation se produire. Les micelles agglomérées se colorent en violet dans mon réactif. La cellule molle, devenue implasmolysable, a perdu son hémiperméabilité ; ses membranes plasmique, nucléaire, vacuolaire, sont désorganisées ; le pH passe de 7,5 à 5. La cryosynérèse irréversible aboutissant au dégel à la coagulation, à la mort, est donc bien la cause qui, dans nos régions, occasionne tant de désastres à l'agriculture.

Quant à la cryosynérèse cytonucléoplasmique réversible, nous avons deux cas à considérer : celui des cellules en vie active contenant encore plus de 12 % d'eau, et celui des cellules en vie ralentie au-dessous de 12 % d'eau jusqu'à ce qu'elles n'émettent plus de vapeur dans le vide. Le premier cas concerne les plantes gelées dans nos régions tempérées et celles qui sont adaptées aux grands froids. Mais, pour que le protoplasma synérésé et non coagulé reprenne son état colloïdal initial nécessaire à la reviviscence, il faut qu'il ait été déshydraté avant le froid ou qu'il ait sécrété dans le suc des vacuoles des substances organiques ou minérales ayant accru leur pouvoir osmotique ; ensuite, il est nécessaire qu'au dégel il reçoive un supplément d'énergie. Ces conditions sont réalisées sur les terres circumpolaires avec un jour et une nuit de six mois chaque, où il y a dessiccation pendant l'automne, gel lent pendant l'hiver, dégel lent en juin. Ainsi, j'ai démontré que des Mousses contenant 60 à 80 % d'eau, fixées au réservoir d'un thermomètre à pentane, mettant 15 minutes à se refroidir lentement jusqu'à — 190°, résistent dans l'air liquide, non seulement 30 secondes, comme dans les expériences de M. LUYET, mais encore 1 heure, 24 heures, 7 jours, si on les retire lentement du cryostat et si, après, on termine leur dégel avec un supplément d'énergie en les chauffant quelques secondes au-dessus d'une flamme. ILJIN, en 1933, avait prouvé que les cellules du chou rouge à 80 % d'eau survivent, si l'on prend la précaution de les geler et dégeler *lentement* dans une solution plasmolysante. Mais pour les cellules avec moins de 12 % d'eau, leur cryosynérèse cytonucléoplasmique est toujours réversible avec *réviviscence certaine après un gel et un dégel lents.* Ces faits m'ont incité à parfaire la cryosynérèse et à la conserver réversible pour une durée indéfinie, en la combinant avec les plus grands froids, la plus extrême déshydratation dans les vides les plus élevés. Des spores diverses, des grains de pollen, des graines décortiquées ont été portées pendant des heures, des jours, des semaines, à — 190°C, — 250°, — 271° et récemment, pour la première fois, à — 273°C, à quelques millièmes près, pendant deux heures. Après gel et dégel lents, tous ces organismes ont survécu, bien que leur activité ait été suspendue pendant une durée presque indéfinie.

L'ACTION DU FROID ARTIFICIEL SUR LES CHAMPIGNONS COMESTIBLES

par Jole Scurti

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Les recherches qui forment l'objet du présent rapport ont été exécutées sur des exemplaires jeunes et sains d'*Amanita Caesarea* et *Boletus edulis.* Des pièces de chaque espèce furent portées lentement à 0° et ensuite maintenues deux mois à — 20°, tandis que d'autres pièces furent congelées rapidement à — 20° C. Nous avons ainsi obtenu, pour chaque espèce de champignons, 2 séries de matériel histologique :

1) Amanite congelée lentement.
2) Amanite congelée rapidement.
3) Bolet congelé lentement.
4) Bolet congelé rapidement.

Au bout des deux mois, les champignons furent inclus à la paraffine à basse température et colorés avec Hématéine et Rouge Congo. Les coupes microscopiques obtenues ont été comparées avec du matériel témoin, et nous avons fait les observations suivantes :

1) *Amanita Caesarea congelée lentement.* Les hyphes de l'épiderme du chapeau sont beaucoup plus homogènes que chez le matériel normal et celles plus profondes sont plus hématéinophiles, beaucoup rétrécies et les lacunes entre elles sont plus larges. Les lames sont fortement rétrécies (environ 1/5 de la largeur normale), avec les éléments du subhymenium intensément colorés et les basidioles plus sphériques avec des amas protoplasmiques. Les hyphes de la trame des lames sont très minces. On remarque de petites altérations dans les éléments du pied.

2) *Amanita Caesarea congelée rapidement.* Les résultats sont à peu près les mêmes.

3) *Boletus edulis congelé lentement.* On remarque dans les hyphes du chapeau une moindre différenciation du contenu cellulaire qui est devenu aussi plus hématéinophile et plus granuleux que dans le matériel normal. Les espaces entre les hyphes sont augmentés par la rupture d'hyphes et la confluence de plusieurs espaces. Les tubes sont rétrécis et les éléments de l'hyménium plus hématéinophiles que ceux des chapeaux normaux. Les hyphes apparaissent plus minces. Dans les éléments du pied les altérations sont moins marquées.

4) *Boletus edulis congelé rapidement.* Résultats semblables à ceux qui concernent les champignons congelés lentement.

Ces expériences ont démontré que les altérations les plus importantes qu'on observe dans les champignons congelés se trouvent dans les tissus périphériques. Ceux-ci en effet présentent une colorabilité des membranes augmentée, une moindre épaisseur des hyphes, une condensation du protoplasme et une disparition de la différence de coloration entre les noyaux et le cytoplasme. Les basidioles tendent à prendre la forme sphéroïdale.

Du point de vue microchimique on observe que le glycogène, qui peut être mis en évidence par le réactif de Schiff après traitement préalable à l'acide chromique, et qui se présente sous forme de granules assez abondants dans l'hymenium soit d'*Amanita* soit de *Boletus*, se dissout dans les champignons congelés.

On peut conclure que, soit du point de vue morphologique, soit du point de vue microchimique, les champignons congelés ne subissent pas de modifications substantielles, ce qui est confirmé par le fait que les propriétés organoleptiques se maintiennent à peu près inaltérées et qu'on ne remarque pas de signes de processus autolytiques.

DIFFERENTS MODES D'ALTERATION IRREVERSIBLE PRODUITS PAR LA REFRIGERATION DANS LES CELLULES RADICULAIRES D'OIGNON (*ALLIUM CEPA*)

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L'apparition de cristaux de glace à l'intérieur d'une cellule lui est en général fatale. Une cellule analogue peut-elle être tuée par le froid sans qu'il s'y forme de cristaux de glace ?

Pour répondre à cette question, nous avons étudié les effets de la congélation sur des racines de bulbes d'Oignon développées normalement dans l'eau, ou dans une solution très diluée de rouge neutre. Des coupes longitudinales à main levée, montées dans l'eau, sont refroidies sous le microscope grâce à un dispositif spécial. Si la température s'abaisse rapidement (en quelques secondes) à — 6° environ, une masse de cristaux réfringents apparaît dans les cellules qui, les unes après les autres, s'opacifient. Les divers constituants de la cellule ne peuvent alors être distingués. Au réchauffement, on retrouve le cytoplasme très contracté, hétérogène, et le noyau structuré et souvent alvéolisé ; les vacuoles ont été détruites. L'observation des coupes fixées au froid et au réchauffement confirme ces remarques. Le chondriome, quelle que soit sa composition initiale, est réduit à des globules, parfois vésiculisés, de tailles variées, et pour la plupart très chromophiles.

Quand le froid s'établit plus lentement (plusieurs minutes pour passer de + 18°C à — 6°), le protoplasme peut être le siège d'une altération soudaine, irréversible. A travers le milieu de montage congelé et transparent, cytoplasme et noyau prennent tout à coup l'aspect d'un liseré sombre, structuré, contracté autour de la vacuole (colorée au rouge neutre) en apparence inaltérée. Au dégel, les colloïdes vacuolaires diffusent à travers le cytoplasme que le froid a désorganisé. Des mitochondries presque exclusives, quelques bâtonnets tous très colorables représentent le chondriome dont l'aspect n'évolue plus, que les fixations aient lieu pendant le froid ou le réchauffement. Les altérations cytoplasmiques sont moins grossières quand les limites des vacuoles semblent inaltérées. Cependant, elles n'en sont pas moins définitives. L'état du noyau est très analogue dans les deux cas, comme celui du chondriome. Selon la vitesse de congélation, le contenu vacuolaire, coloré au préalable au rouge neutre, peut se diviser en un grand nombre de globules fortement teintés ou en un réseau complexe d'un rouge sombre. L'évolution du contenu vacuolaire est variable selon les conditions d'application du froid. Dans tous les cas, les trois phases considérées comme étant la substance vivante de la cellule : cytoplasme, noyau, chondriome, sont désorganisées et nous n'avons jamais observé que l'une le soit sans les deux autres. Chacune d'elles s'altère pourtant d'une façon particulière (alvéolisation, apparition d'agrégats irréguliers plus colorables dans le cytoplasme, vacuolisation et condensation du réseau chromatique, division des chondriosomes).

Ces désordres demeurent intracellulaires, les membranes cellulosiques restent intactes ou à peine déformées.

Tous ces phénomènes sont dus directement à l'action du froid, ainsi que le prouve l'observation des cellules pendant la congélation. Les noyaux se vacuolisent sans que la cellule se déshydrate. Le cytoplasme peut rester régulièrement accolé aux parois cellulosiques, bien que son équilibre colloïdal soit détruit. Dans d'autres cas, on le voit se contracter, mais ce phénomène est soudain, et traduit non pas une déshydratation progressive, mais la rupture brutale de son état physico-chimique normal ; le contenu vacuolaire n'a pas marqué de modification.

En conclusion, on voit que si l'on ralentit suffisamment la congélation, les cellules étudiées peuvent mourir sans que leurs constituants figurés paraplasmiques (contenu vacuolaire, membrane) paraissent altérés. Les désordres n'atteignent alors que la matière vivante au sens strict dont tous les constituants (noyau, cytoplasme, chondriome) sont désorganisés simultanément.

Un séjour suffisant dans un froid moins intense (0°) provoque d'autres altérations qui sont réversibles par retour à la température ambiante. Ces troubles atteignent surtout le chondriome et le cytoplasme. Ils font l'objet d'une communication à la section de Cytologie.

FROSTHARTE UND AUSTROCKNUNGSRESISTENZ BEI IMMERGRUNEN ARTEN DER ALPINEN WALDGRENZE

von A. Pisek

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Einige tropische Arten leiden schon durch Temperaturen knapp über 0°. Weitaus die meisten Pflanzen werden durch Kälte erst bei Frost geschädigt. Am empfindlichsten sind in Entfaltung begriffene Knospen und junge Blätter. Aber auch das ausgereifte Laub der Sommergrünen verträgt im allgemeinen nur wenige Grade unter Null. Nicht viel widerstandsfähiger sind die reifen Blätter der Immergrünen, von denen selbst Arten nordisch-alpiner Verbreitung im Hochsommer bestenfalls — 6 bis — 8 Grad aushalten. Sie alle erfrieren sobald sie gefrieren, d.h. sobald sich Eis in den Geweben bildet. Im Winter hingegen sind die letzterwähnten Immergrünen sehr viel resistenter. Selbst das Laub des als Muster eines Schneeschützlings bekannten *Rhododendron ferrugineum* verträgt bei der Frostprüfung mindestens —15°, *Loiseleuria procumbens* von der Waldgrenze hält bis —35° aus, *Picea excelsa* und *Pinus Cembra* sind noch stärkerer Kälte gewachsen (Pisek und Schiessl, 1947). Im Winter können die Blätter dieser Arten bei tiefem Frost schadlos steif gefrieren, also ohne weiteres starke Eisbildung in den Geweben vertragen. Mag wenig oder viel Eisentstehen, mag die Eisbildung auf die Interzellularen beschränkt bleiden und hier auf Kosten von Zellwasser wachsen oder in die Vakuolen übergreifen : wohl stets wird dadurch direkt oder indirekt dem Plasma Wasser entzogen. Es liegt nahe *darin* mindestens *eine*, wenn schon nicht *die* Hauptursache des Erfrierens von Pflanzengeweben zu sehen. Frosttod liefe also auf kalten Austrocknungstod hinaus (Kessler und Ruhland, 1938). Ein Individuum im frostharten Zustand sollte demnach durch grössere plasmatische Austrocknungsresistenz (Höfler, 1943) ausgezeichnet sein als im frostempfindlichen Zustand. Um dies zu prüfen, schienen die Immergrünen der alpinen Waldgrenze, bei welchen wir in früheren Untersuchungen eine klare Jahresschwankung von Frostanfälligkeit (Sommer) zu ausgesprochener Frostresistenz (Winter) von zum Teil sehr grosser Amplitude gefunden hatten, bestens geeignet (siehe oben).

An der Waldgrenze, 1900 m., wurden mehrmals im Wechsel der Jahreszeiten Zweige von *Pinus Cembra, Picea excelsa, Loiseleuria procumbens, Arctostaphylos Uva-ursi* und *Rhododendron ferrugineum*, aus dem Botanischen Garten solche der atlantischen Spezies *Hedera Helix* entnommen und deren isolierte Blätter bei den jeweils im Freien herrschenden Temperaturen austrocknen gelassen. Im Winter kamen die Blätter zur Beschleunigung ihres Wasserverlustes in Schwefelsäure-Exsikkatoren, die im Freien in einem meteorologischen Wetterhäuschen untergebracht waren. Ein Teil der Blätter wurde in entsprechenden Zeitabschnitten gewogen und aus der Differenz von Frisch- und Endtrockengewicht der jeweilige Wassergehalt ermittelt. Der andere Teil lieferte das Material zur Feststellung der schliesslich eintretenden Schäden. Solche waren nach dem Aufquellen der Blätter im Sommer gewöhnlich schon makroskopisch leicht erkennbar ; im Winter meist schlecht zu beobachten. Deshald wurden im Winter Schnitte angefertigt. Bei *Picea* konnte die Diagnose über Leben oder Tod der Zellen nach Vitalfärbung mit Hilfe der Plasmolyse gestellt werden ; bei den übrigen Arten, welche das Neutralrot im Zellsaft ausfällen, ermöglichte es die Vakuolen-Primärfluoreszenz des Mesophylls den Prozentsatz toter Zellen zu ermitteln (LARCHER, 1954). Wenn der Wassergehalt eines Blattes mehr und mehr sinkt, treten mit Unterschreitung einer gewissen Grenze zunächst ganz geringfügige nekrotische Bezirke von 1-2 % (« kritischer Wassergehalt »), dann zunehmend umfangreichere Schäden auf (10 %, 30 %). Alle untersuchten Arten erreichen im Winter die einzelnen, definierten Schadenstufen bei geringerem Wassergehalt als im Sommer ; alle vertragen im Winter, zur Zeit da sie auf dem Höhepunkt ihrer Frosthärte sind, die stärkste Entwässerung. Mit dem Frühjahr nimmt die Austrocknungsresistenz mit der Frosthärte ab und steigt dieser konform nach dem Sommerminimum wieder an. Der Jahreszeitliche Rhythmus beider Eigenschaften erwies sich bei manchen Arten vorwiegend endogen (resistenzstabile), bei anderen mehr temperaturabhängig (resistenzlabile). Bei *Rhododendron*, das hinsichtlich der Frosthärte ausgesprochen labil erscheint, finden sich unverkennbare Anzeichen, dass dasselbe auch hinsichtlich der Austrocknungsresistenz gilt. Da stets voll ausgereifte Blätter gleicher Exposition von den selben Individuen geprüft wurden, können Zellform, — grösse und — wasserkapazität — kurz alles was bei der Austrocknungsresistenz noch mitspielt — als konstant und die beobachteten jahreszeitlichen Unterschiede als solche der Plasmaresistenz angesehen werden. Die gleichzeitige und gleichsinnige Aenderung von Austrocknungsresistenz und Frosthärte infolge Wandel des inneren Zustandes wie auch durch Wechsel des Wärmeklimas weist eindeutig darauf hin, dass beiden Arten pflanzlicher Resistenz eine gemeinsame plasmatische Komponente zugrunde liegt. Das bedeutet jedoch nicht, dass bei Aenderung dieser plasmatischen Grundlage Frosthärte und Austrocknungsresistenz sich stets beide gleichzeitig im selben Ausmasse ändern müssten. Jede Resistenz umfasst ausser der gemeinsamen Teileigenschaft auch noch andere und hängt von anderen Umständen ab. So ist für die Frosthärte die Geschwindigkeit und der Ort der Eisbildung sowie die Menge des entstehenden Eises mitmassgebend, was bei der Austrocknungsresistenz nicht in Frage kommt.

Résumé. — Les feuilles de diverses plantes alpines présentent de grandes variations de résistance au gel suivant la saison ; le maximum de résistance s'observe en hiver. Des expériences montrent que la résistance des feuilles à la dessiccation passe aussi par un maximum en hiver. Cette résistance varie suivant un rythme saisonnier endogène ou lié aux variations de température. La coïncidence observée entre la résistance au gel et à la sécheresse n'est cependant pas totale ; ainsi, les conditions de formation de la glace interviennent dans la résistance au gel.

DIE KALTERESISTENZ MEDITERRANER IMMERGRÜNER HOLZPFLANZEN

von Walter LARCHER
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Die Kälteresistenz der Assimilationsorgane immergrüner Holzpflanzen von der Nordgrenze des mediterranen Florengebietes wurde zu verschiedenen Jahreszeiten experimentell ermittelt. Die stets von denselben Individuen stammenden Proben wurden hiezu locker in Glasgefässe eingefüllt und diese in der von ULMER (1937) und PISEK und SCHIESSL (1947) geübten Weise durch Untertauchen in einer auf die gewünschte Temperatur eingestellten Eis-NaCl- oder Eis-CaCl_2-Kältemischung langsam gekühlt. Die Lufttemperatur an verschiedenen Stellen im Innern der Frostkammern (Kammerrand, -mitte, -boden) und gelegentlich auch unmittelbar die Blattemperatur wurden mit Hilfe von Thermoelementen in Verbindung mit einem sehr empfindlichen Spiegelgalvanometer gemessen. Nach langsamen Auftauen der Proben und Einwässern geben sich Kälteschäden im Sommer längstens nach Stunden, im Winter meist ebenfalls rasch, bei manchen Arten jedoch (*Cupressus, Arbutus*) erst nach Tagen eindeutig als Verfärbungen zu erkennen. In seltenen Zweifelsfällen (assimilierende Sprosse von *Citrus trifoliata,* Triebe von *Cupressus sempervirens*) schaffte fluoreszenzmikroskopische Untersuchung (Vakuolenprimärfluoreszenz-Test, LARCHER, 1954) rasch Klarheit.

Im *Winter* (Jänner) vertragen vollständig ungeschädigt rund dreistündigen Frost.

von —5°C	Blätter und Sprosse von *Acacia dealbata,*
—8° bis —9°	Blätter von *Rosmarinus officinalis* u. *Nerium Oleander,*
—9° bis —10°	Blätter von *Laurus nobilis* u. *Arbutus Unedo,*
—11° bis —12°	Nadeln von *Pinus Pinea,* Blätter von *Viburnum Tinus, Olea europea* und *Chamaerops humilis,*
—12°	Blätter von *Trachycarpus excelsa* u. *Quercus Ilex,*
—15°	Triebe von *Cupressus sempervirens,*
—16°	Nadeln von *Cedrus deodara* und *Cedrus atlantica,*
—18°	Assimilationssprosse von *Citrus trifoliata.*

Nach weiterem Absinken der Versuchstemperatur um ein Grad stellen sich allgemein erste Schäden (höchstens 5 % tot, Rest gesund) ein.

ULMER (1937) und PISEK und SCHIESSL (1947) hatten an immergrünen alpinen Pflanzen eine erhebliche Abnahme der Frosthärte vom Winter zum Sommer gefunden. Auch die Kälteresistenz immergrüner Holzpflanzen aus dem klimatisch ausgeglicheren, wärmeren Mittelmeerraum pendelt innerhalb des Jahres zwischen zwei Extremwerten, einem winterlichen Frosthärtemaximum und höchster Frostempfindlichkeit im Sommer, deren Abstand artabhängig verschieden ist :

Im Sommer sind empfindlicher als im Winter

um 2°C	*Chamaerops, Trachycarpus,*
um rund 5°bis 6°	*Laurus, Arbutus,*
um 6°	*Citrus*-Sprosse
um rund 6° bis 7°	*Pinus, Olea, Quercus, Viburnum,*
um 8° bis 9°	*Cupressus.*

Besonders fällt das Verharren der beiden Palmen auf hoher Resistenzstufe auch während des Sommers auf.

Bei einer Temperatur von —7 bis —8°C in der Kühlkammer im Winter, spätestens um —7°C im Sommer, sind die Blätter aller Versuchspflanzen regelmässig steif gefroren. Ein wesentlicher Grund für die grössere winterliche Kälteresistenz mag also bei den meisten Versuchspflanzen in der höheren Widerstandsfähigkeit der Zellen gegen Schädigung durch Eisbildung in den Geweben zu suchen sein. Im Sommer hingegen fällt offenbar der Erfrierungstod mit dem Zeitpunkt der Eisbildung zusammen.

An *Pinus Pinea, Cupressus sempervirens, Laurus nobilis, Quercus ilex, Arbutus Unedo* und *Olea europea* wurde in Anlehnung an die Methode von PISEK und SCHIESSL (1947) untersucht, ob und wie weit Pflanzen im Winterzustand durch fünftägigien Aufenthalt im warmen (+ 20°C) Zimmer verwöhnt, durch fünftägiges Kühlen bei —6°C abgehärtet, sowie durch ungefährliches Trocknen beeinflusst werden können. Die Frosthärte der mediterranen Versuchspflanzen liess sich im *Winter* durch Frostbehandlung zwar kaum weiter treiben, durch Verwöhnen jedoch kräftig senken. Ergebnisse von Kühlversuchen an vergleichsweise herangezogenen Exemplaren, die im Gewächshaus des Botanischen Institutes in Innsbruck warm überwintern, decken sich mit den an verweichlichtem Material erhaltenen völlig. Sommerliche Empfindlichkeit wird im Winter allerdings in keinem dieser Fälle erreicht, obzwar Anzeichen (Schadenbild u. a.) darauf hinweisen, dass die Schäden bei verweichlichtem Material im allg. unmittelbar auf Eisbildung zurückzuführen seien. Im *Sommer* steigert Frostbehandlung die Kälteresistenz nicht, wohl aber erfrieren — wie zu erwarten — leicht angetrocknete Blätter später als wasserreiche (Gefrierpunktserniedrigung durch passiv erhöhte Zellsaftkonzentration).

Aus der Jahresschwankung der Kälteresistenz und deren Beeinflussbarkeit, aus Schadenbild und aus einzelnen orientierenden Bestimmungen des osmotischen Wertes, die nur mässige oder gar keine Zunahme, ja sogar Abnahme des osmotischen Wertes vom Sommer zum Winter anzeigen, wie auch unter Berücksichtigung der Beobachtungen von BRAUN-BLANQUET u. WALTER (1931) über den Jahresgang des osmotischen Wertes von Mittelmeerpflanzen, kann geschlossen werden, dass an der Steigerung der Kälteresistenz zum Winter auch bei mediterranen immergrünen Holzpflanzen eine Zunahme der plasmatischen Resistenz (plasmatische Abhärtung) massgeblich beteiligt ist.

Résumé. — Des feuilles et des pousses vertes d'arbres et arbustes méditerranéens toujours verts furent examinées en diverses saisons en les laissant geler à des températures constantes, comprises entre —3°C et —20°C, dans des petites chambres frigorifiques, pendant trois heures. La résistance au froid varie au cours de l'année, selon les espèces, avec une amplitude spécifique, entre la sensibilité maximale au froid pendant l'été, et d'autre part la résistance maximale pendant l'hiver. La grande résistance hivernale peut être presque annulée en sensibilisant les rameaux dans un endroit bien chanuffé. L'augmentation de la résistance au froid jusqu'à l'hiver semble dépendre en premier lieu de l'endurcissement du protoplasme.

THE ROLE OF CARBOHYDRATES IN FROST RESISTANCE

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It has long been known that sugars increase when a plant becomes frost hardened and that the varieties of a species frequently show an almost perfect correlation between sugar content and relative frost resistance (e. g. AKERMAN, table 45, p. 190). This relationship has been found by so many workers in so many lands, and for so many plants, that it cannot possibly be accidental. On the other hand, it has been just as conclusively proved by numerous investigators that sugar content can increase without any accompanying increase in frost resistance (e. g. potato tubers kept at 0-5°C), and that frost resistance can increase without any accumulation of sugars (e. g. the late fall increase in many plants). We are thus faced with the dilemma of trying to explain why sugars may sometimes have a pronounced effect on hardiness, while at other times they have no effect.

It seems obvious from these facts that sugars must be a secondary factor. LEVITT (1941) suggested that they exert their protective effect only when superimposed on a primary protoplasmic change. The possibility was also raised that the starch-sugar conversion may conceivably remove an injurious effect of the hard starch grains on the protoplasm in the contracted (frozen) cell. SIMINOVITCH et al. (*in press*) have recently championed this second view.

That a protective effect of a starch — sugar conversion cannot always be involved is obvious from the fact that the sugar accumulation during hardening is frequently directly due to photosynthesis and starch does not occur in either the frost-sensitive or the frost-hardy plants. It is, in fact, just such plants (e. g. grains) that show the most striking correlation between sugar content and hardiness.

As an extension of explanation 1), the following hypothesis is proposed. When frost hardiness is correlated with sugar content, it is not the free sugars themselves, but a sugar-protein complex (*i. e.* a mucoprotein) that is directly responsible for the hardiness. Due to mass action, an increase in free sugar content would be accompanied by an increase in mucoproteins or in the amount of sugar associated with each protein molecule. Only those plants capable of forming such mucoproteins would be able to increase in hardiness with increased sugar content.

To investigate this hypothesis, cabbage seedlings were hardened for 10 to 14 days under continuous lights at 5°C and their proteins then compared with those of similar seedlings from the greenhouse. This method has always been found to cause a marked increase in frost resistance — the hardened plants being able to survive about — 6°C, the unhardened being killed by about — 2°C (LEVITT, 1939). Though no direct tests of hardiness were made in these experiments, the appearance of the hardened plants, their high osmotic potential, and the superior deplasmolysis resistance always indicated superior hardiness in the greenhouse plants.

The proteins were extracted and fractionated according to a scheme previously used for potato tubers (LEVITT, 1952). The protein fractions were then analyzed for total carbohydrates by the anthrone method. The results were as follows :

TABLE 1

Carbohydrate contents (%) of protein fractions of cabbage seedlings and potato tubers. Each value an average of 4-10 determinations.

	Cabbage	seedlings	Potato tubers	
	Unhardened	Hardened	10 days at 26°C	3°C
Mitochondria			8.3	10.2
Microsomes	17.5	17.1	6.8	9.8
Acid-insoluble proteins	5.7	6.1	5.0	6.0
Globulins	10.8	8.4	4.5	3.9
Albumins	28.8	22.3	6.7	6.0

There is obviously no evidence of a higher carbohydrate content in the hardened cabbage. The microsomes and acid-insoluble proteins show no difference, whereas the globulins and albumins actually have a higher carbohydrate content in the unhardened plants. Yet it is interesting to note that with the exception of the acid-insoluble proteins, the fractions all show a markedly higher sugar content that in the case of the potato tuber which is incapable of developing any frost resistance whatever. It may be suggested that this initially high sugar content of the proteins is essential for the plant to develop any frost resistance, and that other factors may then be superimposed on this. However, if this were true, the role of the sugars is not likely to be as a preventative of protein denaturation, since even the potato albumins with their low sugar content are not denatured by boiling in M citric acid. It is interesting to note that the acid-insoluble proteins have the same sugar content in both cabbage and both potato series.

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NON-FREEZING INJURY IN PLANT TISSUES WITH PARTICULAR REFERENCE TO THE DETACHED PLUM FRUIT

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Low temperature injury occurring at temperatures above the freezing point of plant tissue is very different in nature from that occurring at temperatures below the freezing point. The killing of the cells is attributable not to drastic change of physical state but rather to metabolic disturbance, the degree of which is often a function of the duration of the period of exposure to low temperature.

This type of injury is one about which there is very little fundamental knowledge.

Low temperature injury once it becomes manifest can be recognised by the appearance of regions in the tissue that are either morbid or already dead. Though a critical test for the transition from life to death of the cell is lacking, an early symptom of morbidity is often the gradual filling up of the intercellular spaces by the cell fluids. A brown discolouration may already be present when the tissues are cut open or it may appear quickly upon access of oxygen through the cut surface.

Observations have usually been made on the effect of temperature and duration of exposure upon the extent and frequency of occurrence of injury in comparable samples of fruits. Typical curves for the fruit of the Victoria plum are shown. (Fig. 1. Low Temperature Injury Curves — Victoria Plum.) These curves reveal the interesting fact that though an increasing number of plums are injured with extension of the period of exposure, injury reaches an earlier maximum at intermediate temperatures. In point of fact, although not shown in this graph, the total number of plumes ultimately affected may be greater at the lower temperature than at the intermediate temperature.

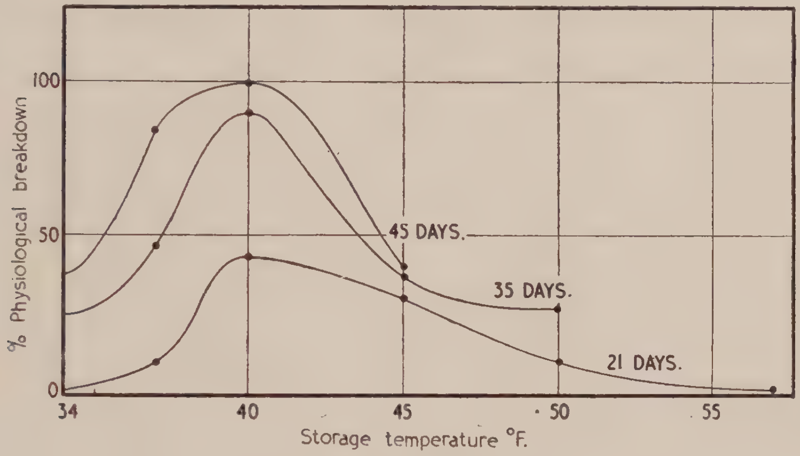


Fig. 1.

An interesting and ingenious hypothesis to explain this relationship has been put forward by **Plank**. **Plank** pictures low temperature injury as the result of the accumulation of cell-toxin (Zellgift), arising from a disturbance of the normal run of biochemical processes which is conditioned through the different degrees of retardation of the individual inter-connected chemical reactions with decreasing temperature. Whether a given fruit is injured or not at a given temperature depends upon whether the rate of building up of the toxin (Zellgift Bildung) exceeds the rate of dispersal (Zellgift Veratmung) at that temperature. Below the critical level at which the rate of building up exceeds the rate of dispersal then there is a gradual accumulation of the toxin until such time as it reaches a lethal level and injury to the cells becomes manifest. By assigning arbitrary values for the temperature coefficients of the two individual reactions governing the building up and the dispersal of the toxin, the critical temperature at which the reactions cancel each other, their range of variation and the limiting value of the accumulated toxic material in the fruit, Plank has been able to produce curves which correspond very closely in form to those found experimentally by Rees Davies and his co-workers in S. Africa and to those by Smith in England.

The curves for low temperature injury based on observations made on the Victoria plum introduce a complication. (Fig. 2. Internal Browning and Jellying Curves for the Victoria Plum.) Two symptomatically distinguishable types of injury have been found. The one is characteristic of exposure to temperature below 37°F. (2.8°C) and manifests itself as a brown discolouration of the tissues round the stone usually appearing as radiating streaks in the early stages, but in advanced stages affecting more of the flesh of the fruit. The other type of injury known as « Jellying » occurs during storage at temperatures above 34°F. (1°C) and manifests itself most rapidly in the range 40°-45°F. (4.4°-6.7°C). It also appears when plums which have been exposed to temperatures below 34°F. (1°C) for a sufficient length of time are removed to a higher temperature.

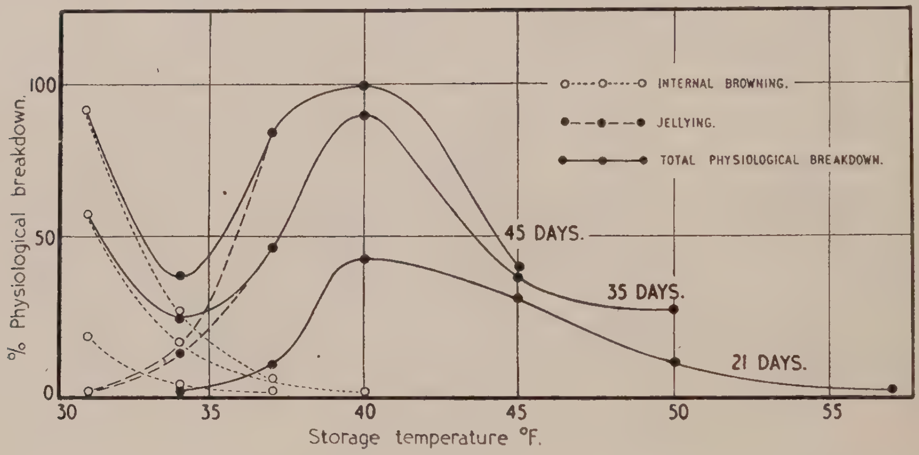
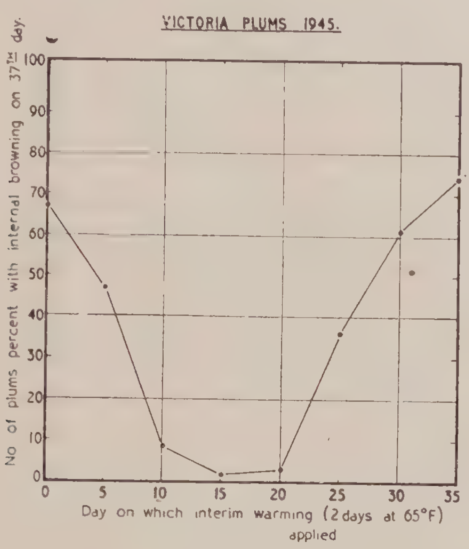


Fig. 2.

Thus for Victoria plums a period of exposure of 21 days at 31°F. (—0.5°C) is usually sufficient to bring about visible injury in some proportion of a sample of fruits. If however, the fruits are warmed at about the seventeenth day to 65°F. (18.3°C) for a period of 1-2 days, then when examined after a further 21 days of exposure to 31°F. (—0.5°C) it is found that these plums are free from injury. (Fig. 3. Interim warming and Internal Browning in the Victoria Plums.)

On the basis of PLANK's hypothesis some such relationship might be expected since the raising of temperature should assist dispersal of the toxin provided it is sufficiently volatile.

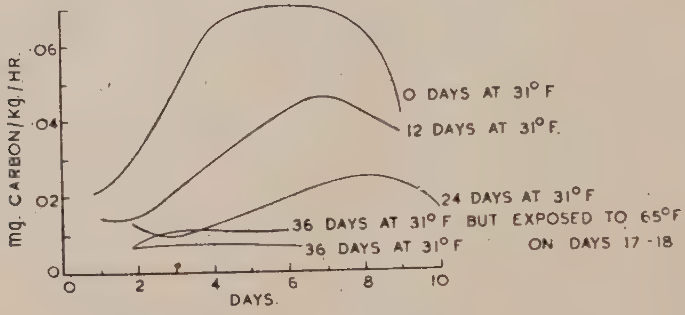


The effect of interim warming treatment 65° F. (18·3°C.) on development of internal browning in plums stored at 31° F. (-0·5°C)

Fig. 3.

The total volatile production of Victoria plums has been measured by passing the CO_2-free air stream from a sample of the fruit over copper gauze in an electric furnace. Estimation of the resulting carbon dioxide production after absorption in a solution of sodium hydroxide gives a measure of volatile products including ethylene. At a temperature of 65°F. (18.3°C there is a characteristic rate-time curve with a distinct peak when production is measured over a period of seven days. Measurement of this volatile production on removal to 65°F. (18.3° C) by samples exposed for increasing periods of time at low temperature 31°F. (—0.5°C) shows that while there is still the characteristic peaked curve, the level and consequently the total output of volatile substances diminishes. (Fig. 4. Volatile production at room temperature after exposure of the Victoria Plum fruit to low temperature). It is therefore possible that with the passage of time at low temperature an increasing amount of volatile substances remains in the tissues and can no longer be dispersed on warming. If however, warming is applied before the lapse of a critical period then there is a dispersal of some of these substances and a further period of time is required for them to reach a harmful concentration in the tissues.

A preliminary attempt has been made to obtain evidence as to whether ethylene and carbon dioxide in the plum fruit contribute to low temperature injury.



PRODUCTION OF VOLATILE SUBSTANCES AT 60° F.

Fig. 4.

There is thus evidence that the presence of ethylene, carbon dioxide and oxygen can influence the incidence of low temperature injury. The controlling influence of oxygen on jellying is clearly demonstrated.

PHYSIOLOGICAL DIFFERENCES IN CONIFER LEAVES OF DIFFERENT AGES

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In comparing the ability of different species of conifers to withstand certain factors of their environment such as drought and freezing, one is confronted with great differences in leaves of different ages. While these differences are quite pronounced in *Pinus ponderosa,* they are not so pronounced in *Abies grandis* and *Pseudotsuga taxifolia,* although the trends with increasing age are the same in all species studied.

Respiration rate determinations with a NaOH-absorption apparatus of excised leaves showed differences among leaves of various ages of *Pinus ponderosa.* This amounted to about 13 μ g / min. / g. fresh weight at 22°C in one year old leaves, 10 in 2 year-old, and 9 in 3 year-old. Speed of air flow was 3 liters per minute and leaves were bunched together in a glass tube, about 4 grams of leaves (fresh weight) per test sample. Results for *Abies grandis* showed only 1 μ g / min. / g. F. W. but no appreciable differences could be detected in rates among 1, 2, and 3 year-old leaves.

Leaves immersed in a 1 % solution of 2,3,5 triphenyl tetrazolium chloride unbufferred and not O_2-free showed much more rapid appearance of reduced formazan in 1 year-old than older leaves. Not so much difference was shown in ages of 2 to 5 years old, although a trend to slower and weaker reaction with increasing age in a given time was evident in all three species. This also suggests a higher respiration in young leaves.

Freezing studies in a specially designed cold chamber using a 1/3 H.P. compressor and low temperature freon showed that even in late summer and early autumn excised 1 year-old leaves were much less able to withstand slow cooling and thawing (1°C/hr.) than older leaves. This difference amounted to about — 7°C killing temperature for the youngest leaves and about — 15°C for the 2 year-old leaves. This seemed to be true of all species studied. In mid winter when leaves were brought in from sub-zero temperatures, cooling of all ages of leaves to — 60°C by the slow method mentioned above resulted in no appreciable damage. Rapid warming 10°C / min.) in mid-winter was found to be more harmful than slow warming, and rapid cooling (same rate) was also found to be harmful if leaves had been exposed to supra-freezing temperatures for periods of about 24 hrs, depending on weather conditions.

Desiccation studies of leaves removed from the twigs and left to dry on the laboratory table showed that the youngest leaves of *P. ponderosa* survived the longest as judged by the tetrazolium test, 2nd year leaves next longest, 3rd next, and so on through 5 years of leaves. Weighing of leaves at 24 hour intervals showed that this was largely a result of slower rates of drying in the younger leaves. Other experiments suggest that the slower drying is a result of more complete stomate closure. Other factors enter in to the survival time, such as initially higher water contents in younger leaves, and ability of younger leaves to dry out to lower moisture contents without dying.

Spectrographic analysis of leaves of various ages in a Wardsworth grating spectrograph showed marked differences between 1 and 3 year-old leaves. Leaves were ashed, packed firmly into hollowed carbons, and arced for 10 seconds. The following elements showed an increase from 1 to 3 year old leaves of *Pinus ponderosa* : Sb, Mn, K, B, Bi, Si, and Al. No change was noticed in amounts of Ca and Mg. Results for Cu were indefinite.

THE MECHANISM OF VERNALISATION WITH PARTICULAR REFERENCE TO THE TEMPERATE CEREALS

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In 1937 we presented a purely hypothetical schema to accommodate the effects of light and temperature on flowering in cereals as then known. These facts were- (1) Spring cereals are long day plants ; in short days flower differentiation is slow and ear emergence fails. (2) Winter cereals, fully vernalised, behave in a similar manner. (3) In unvernalised winter cereals flowers are differentiated very slowly in long days, and somewhat more quickly in short days ; for ear emergence, however, long days are requisite. (4) The curve relating duration of vernalisation and flowering response is sigmoid in form. In winter rye, for example, the maximum slope occurs at four weeks, and the slope diminishes to a limit at about ten weeks. At this limit the flowering response approximates that of spring rye.

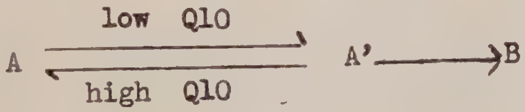
The schema represented the vernalisation process as a chain of reactions, favoured by low temperature, from a precursor A to a substance B. Flowering depends on further reactions from B which are determined by the level of B and by the length of day. In winter rye, unlike some grasses, the cold requirement is not obligate ; presumably some B is present in the unvernalised grain, and increases in the plant until a critical level is reached. There is further evidence for the increase of B in the growing plant : high nitrogen supply increases the number of ears, but in no way retards flowering.

We are here concerned only with the formation of B from the precursor A. The sigmoid form of the « duration » curve indicates that some part of the chain of reactions is autocatalytic. This implies that the limit to the degree of vernalisation possible is imposed by the depletion of A. The reaction A to B depends on low temperature ; its velocity is optimal and constant between 2°C and 7°C, and it ceases at a neutral temperature approximating 12°C-15°C. Above this temperature some annulment of the vernalised condition may occur. This « devernalisation » increases with rising temperature (Q10 about 3.5), but diminishes as the degree of vernalisation increases, until after complete vernalisation high temperature is without effect. In 1947 after 4 weeks vernalisation 3 days at 35°C reduced the effect of low temperature by 55 %, and after 8 weeks, by only 3 %.

To account for devernalisation and for the progressive stabilisation of the vernalised condition mentioned above, the reaction A→B may be assumed to comprise two stages. The first is the formation of a thermolabile intermediate substance A' which is substrate for the formation of the thermostable B. After partial vernalisation, A' will, at the neutral temperature, continue to form B so that the degree of vernalisation depends upon both A' and B. This explains the stabilising effect of a period at the neutral temperature immediately following vernalisation which has been demonstrated by us for rye and also for *Hyoscyamus* by MELCHERS and LANG. At high temperatures, however, A' disappears so that the degree of vernalisation is dependent now only on the amount of B already formed.

The low optimal temperature for formation of B can be attributed to the differential temperature coefficients of the three reactions concerned. That of A→A' is low, that for the inactivation of A' much higher, and for the reaction A'→B intermediate. The resultant reaction at low temperature will then be towards A' and B while at high temperatures the removal of A' will predominate.

The nature of the devernalisation reaction may be deduced from the following considerations. After devernalisation a further period at low temperature fully restores the vernalised condition. The apparent autocatalytic nature of the overall reaction suggests that A is limited in amount, and it thus follows that the disappearance of A' may lead to the re-formation of A. The schema should then be



$$A \underset{\text{high Q10}}{\overset{\text{low Q10}}{\rightleftarrows}} A' \longrightarrow B$$

This need not be the case if A is present in large excess. It has been shown that heating the imbibed grain *before* vernalisation leads to a retardation in flowering, but unvernalised grain is not devernalised by heat. It therefore appears that although no A' is present before vernalisation begins, devernalisation can occur at this stage. The alternative explanation may be the formation of an inhibitor at high temperature which inactivates A' as it is formed. If an inhibitor is formed, however, it does not accumulate since winter rye can be vernalised at any stage of growth. To test whether the precursor A is present in excess the following experiment was carried out. Winter rye was subjected alternately to 2 days at 2°C and one day at 25°C or 30°C, for prolonged periods up to 8 weeks. From previous experience of such treatment A' should be destroyed during each heat period ; the precursor should thus be eliminated and vernalisation made impossible.

The result of this experiment will be presented at the Congress, but the necessary dissections are as yet incomplete. It is, however, already evident that this drastic treatment by no means eliminates the response to subsequent vernalisation. Provisionally, therefore the schema may be presented in the form given above.

So far, the reaction A'→B has not been dealt with. It is known that exposing grain to temperatures of 20°C and 25°C for varying periods changes the response to vernalisation to differing degrees. With short exposures *devernalisation* is found ; with longer exposures flowering is accelerated. It is supposed that reversal (A'→A), occurs as well as the promoting reaction A'→B. In addition the self-perpetuation of B (autocatalysis) takes place and the final effect thus depends on the velocities and temperature coefficients of these reactions.

VERNALISATION UND BLATTBILDUNG

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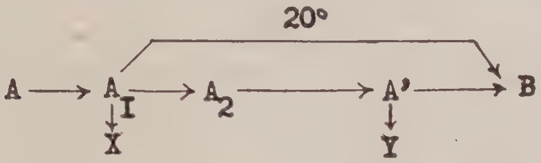
Purvis und Gregory veröffentlichten 1937 ein Schema, das den Zusammenhang zwischen Vernalisation, Photoperiodismus und Blattbildung zu veranschaulichen sucht. Diese Formel wurde 1952 in der Weise ergänzt, dass sie nunmehr sechs Glieder umfasst :

$$\begin{tikzcd}
 & & & E & \\
A \arrow[r, shift left] & A' \arrow[l, shift left] \arrow[r] & B \arrow[ur] \arrow[dr, shift left] & & \\
 & & & C \arrow[ul, shift left] \arrow[r] & D
\end{tikzcd}$$

Hierbei bedeutet A eine Vorstufe, A' eine thermolabile Zwischenstufe und B die thermostabile Endstufe des Vernalisationsprozesses. C und D symbolisieren Substanzen, die unter günstigen photoperiodischen Bedingungen gebildet werden und für die Blütenbildung verantwortlich sind, und E ein unter ungünstigen photoperiodischen Verhältnissen gebildetes Blattbildungshormon. Die Alternative zwischen B → C und B → E wäre demnach als eine Art Kippvorgang aufzufassen, über dessen Ausgang die jeweiligen photoperiodischen Bedingungen entscheiden.

Im Jahre 1953 wurde nun eine Reihe von Vernalisations-, De- und Revernalisations-Versuchen mit der winterannuellen Rasse « *Stockholm* » von *Arabidopsis thaliana* durchgeführt, die von Laibach (1943) für Untersuchungen zur Physiologie der Blütenbildung als besonders geeignet erkannt worden war. Die Ergebnisse dieser Versuche führten einmal zur Einfügung zweier neuer Glieder in die bisherigen Schemata des Vernalisationsprozesses von Lang und Melchers (1947) und von Gregory und Purvis (1952), welch letzteres nunmehr folgendermassen aussehen würde (vgl. Napp-Zinn 1953) :



A_1 stellt ein thermolabiles, A_2 ein thermostabiles Zwischenprodukt des Vernalisationsvorganges dar; X und Y entstehen aus A_1 resp. A' bei 30°C (sonstige Bezeichnungen wie vorher).

Zum anderen lieferten diese Versuche einige interessante Resultate bezüglich der Blattbildung, auf die wir hier kurz eingehen möchten. Die Tatsache, dass bei der Zerstörung der Vernalisations-Zwischenstufen A_1 und A' durch Wärme nicht nur die Zeit bis zur Blüte verlängert, sondern im allgemeinen auch die Blattzahl gesteigert wurde, hätte zunächst die Annahme nahelegen können, dass die (hypothetischen) Devernalisationsprodukte X und Y etwas mit der Blattbildung zu tun haben und sich daher in ähnlicher Weise an die zur Blütenbildung führenden Vorgänge anschliessen könnten wie das Produkt E in dem Schema von Purvis und Gregory.

Nun bewirkt aber eine Wärmebehandlung durchaus nicht immer eine Steigerung der Blattzahl, wie das Ergebnis des folgenden Versuches zeigt : Samen der winterannuellen *Arabidopsis*-Rase *Stockholm* wurden bei 20° zum Keimen gebracht ; die aus ihnen hervorgegangenen Pflanzen blieben bei dieser Temperatur in einer Licht-Klimakammer bis zur Blütenbildung, die durchschnittlich nach 107,8 Tagen eintrat. In dieser Zeit waren 77,6 Rosettenblätter und 15,3 Stengelblätter gebildet worden. Andere Pflanzen der gleichen Rasse wurden nach der Aussaat zunächst 2 Tage bei 20°, dann 5 Tage im Dunkeln bei 30° und schliesslich zusammen mit den Pflanzen der ersten Gruppe bei 20° gehalten. Die Pflanzen dieser zweiten Gruppe blühten durchschnittlich nach 117,2 Tagen und hatten 71,3 Rosetten- und 13,3 Stengelblätter entwickelt, m. a. W., die Blütenbildung war im Vergleich zur Kontrolle um 9,4 Tage verzögert, die Zahl der Rosettenblätter dagegen um 6,3 und die der Stengelblätter um 2,0 gesenkt ($P < 0{,}0002$). — In einem Parallel-Versuch, in dem die 5tägige Wärmebehandlung aber nicht 2, sondern 8 Tage nach der Aussaat begonnen hatte und bei dem wohl gleichfalls nur das erste thermolabile Zwischenstadium (A_1) erreicht worden war, waren dagegen das Blühalter um 28,0 Tage und die Zahl der Rosettenblätter um 10,0 im Vergleich zur Kontrolle gesteigert. Es erschien daher schwer erklärlich, wieso das Wärmebehandlungsprodukt X die Blattbildung im einen Falle hätte hemmen, im anderen hätte fördern sollen ;

ein solcher Zusammenhang zwischen Vernalisation, Devernalisation und Blattbildung ist daher wenig wahrscheinlich.

In einem anderen Experiment wurden Samen nach 1 tägiger Vorquellung bei 20° 28 Tage lang bei +4° vernalisiert und dann bei 20° gehalten ; eine andere Gruppe wurde 30 Tage bei +4° vernalisiert und anschliessend 7 Tage bei 20°, dann 5 Tage bei 30° und von nun ab wieder bei 20° gehalten. Die Wärmebehandlung hatte keinen devernalisierenden Effekt ; die Blütenbildung trat daher bei beiden Gruppen nach rund 56 Tagen (vom Ende der Vernalisation ab gerechnet) ein ; die Zahl der Rosettenblätter betrug aber bei der ersten Gruppe 48,7, bei der zweiten dagegen nur 31,4, war also um 17,3 vermindert ($P < 0{,}0002$).

Diese Befunde gaben Anlass, die Beziehung zwischen Blattzahl und Blühalter in möglichst weitem Rahmen zu verfolgen. Dabei ergab sich ganz allgemein, dass die Blattzahlen bei wärmebehandelten Pflanzen durchweg um etwa 10-20 niedriger lagen als bei nicht wärmebehandelten Pflanzen gleichen Blühalters, ganz unabhängig davon, ob die Wärmebehandlung kurz nach der Aussat der Samen, in unmittelbarem Anschluss an eine 2-10wöchige Vernalisation bei —3,5° bis +4° oder nach einer 1wöchigen « Stabilisation » des Vernalisationseffektes bei 20° stattgefunden hatte, und auch unabhängig davon, ob der Wärmebehandlung eine Kältebehandlung (Revernalisation) folgte oder nicht. Im Hinblick auf die eingangs erwähnten Schemata bedeutet dies, dass mindestens vom Stadium A_1 an bis über das Stadium B hinaus ein wärmeempfindlicher Blattbildungsfaktor anzunehmen ist. Da sicherlich bei einer 30°-Behandlung ein guter Teil des für die Blattbildung geeigneten organischen Materials (Kohlenhydrate, Eiweissstoffe) dissimiliert wird, möchten wir für die Verminderung der Blattzahl wärmebehandelter *Arabidopsis*-Pflanzen gegenüber unbehandelten Pflanzengleichen Blühalters vor allem trophische Faktoren annehmen. In dieselbe Richtung weist auch der allbekannte Einfluss des Bodens auf die Blattzahl. Wir sehen daher in der Blattzahl eine Funktion der trophischen Verhältnisse und der bis zur Blütenbildung verstreichenden Zeit.

VERNALISATION ET FORMATION DES FEUILLES

Résumé. — Les recherches antérieures ont permis d'établir des schémas hypothétiques relatifs à l'intervention de facteurs successifs expliquant les relations entre vernalisation, photopériodisme et développement des feuilles. L'auteur apporte de nouvelles données expérimentales relatives à *Arabidopsis Thaliana* traité dans diverses conditions de température (vernalisation suivie de chauffage à des températures variées). Les nombres de feuilles des plantes traitées par la chaleur sont plus faibles (à évolution florale comparable) que ceux des plantes non chauffées. On doit donc admettre l'existence d'un facteur de formation des feuilles sensible à la chaleur ; des facteurs trophiques doivent aussi intervenir.

LA JAROVISATION DE LA BETTERAVE, DU NAVET FOURRAGER, DU COLZA ET DES CHOUX

par D. Kloen
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La production de la graine de betterave est une culture bisannuelle aux Pays-Bas. Elle demande beaucoup de travail, les planchons constituant des foyers d'infection pour la jaunisse, et pendant les hivers rigoureux il y a souvent des pertes considérables. Toutes ces considérations ont inspiré des recherches sur la possibilité de la production de graines dès la première année à l'aide de semences jarovisées. Il importe que toutes les graines des glomérules soient amenées à germer, qu'elles soient soigneusement traitées pendant la jarovisation et qu'elles soient semées de bonne heure au printemps. Le séchage de la semence trempée favorise considérablement la germination.

Les premières plantes montant à graines s'observent quelques semaines après la montaison des racines qui ont passé l'hiver. Ces dernières montent plus régulièrement. Les plantes jarovisées ne produisent, en général, qu'une seule hampe florale, ce qui fait que la production de graines est moins abondante qu'en culture normale. On essaye d'augmenter la productivité par certains soins culturaux. Le tableau suivant donne les résultats obtenus en 1950 avec de la semence jarovisée pendant 32 jours et semée le 7 mars :

Variété	% de montée
Kuhn P	67,3
Friso	72,4
Eureka	84,7
Ovana	87,9

La proportion de plantes qui produiront de la semence est de 10 à 20 % plus basse.

On a obtenu de bons résultats avec les variétés qui montent à graines facilement. La graine des plantes qui montent de bonne heure est de qualité excellente. Dans le matériel traité, un changement héréditaire se produit peut-être, puisque certaines plantes ne montent pas à graines. Jusqu'ici, les avantages de la jarovisation sont d'intérêt plus direct pour l'obtenteur que pour le cultivateur. Elle est d'importance pour la multiplication rapide de populations hybrides et des familles.

Chez les betteraves, les obtenteurs s'efforcent d'augmenter la résistance à la montée à graines. Parce que les obtenteurs ne peuvent pas toujours semer très tôt au printemps. On peut utiliser de la semence jarovisée qui produit encore assez de plantes montées à graines quand le semis s'est fait tard, ce qui permet une bonne sélection pour la résistance à la montaison.

Variété	Epoque du semis			
	9-3-48		22-3-48	
	jarovisation de 34 jours	pas de traitement	jarovisation de 34 jours	pas de traitement
Hilleshög	36,7	10,1	28,9	4,4
Corona	57,4	15,1	35,2	6,4

L'expérience suivante prouve que des changements héréditaires se produisent. En 1951, de la semence jarovisée fut semée sur de petites parcelles isolées et les graines des plantes montées furent récoltées. En 1952, on obtint aussi de la semence de betteraves qui étaient restées à l'état végétatif en 1951.

En 1953, semis le 17 mars, la montaison était comme suit :

Variété	semences provenant de plantes montées	semences provenant de planchons	semences du commerce
Hilleshög	18,0	2,5	5,2
Avana	29,5	2,2	25,3

Aux Pays-Bas, environ 80.000 ha de terres sablonneuses sont consacrés à la culture du navet fourrager.

La culture grainière courante est bisannuelle, mais pendant les hivers rigoureux beaucoup de plantes qui restent sur les champs sont perdues, parce qu'elles ne supportent pas une forte gelée. Ce matériel, mis en silo, risque de se gâter pendant les hivers doux si la température monte au-dessus de 5°C. Si des graines germées sont soumises au froid pendant une courte période, toutes les plantes montent en graine quand la semence traitée est semée début mars. Les rendements sont suffisants avec un semis en terrains fertiles et un traitement soigné. La graine mûrit à temps pour être utilisée pour la culture de navets la même année.

Plusieurs variétés de colza, qui ne supportent pas les fortes gelées, périssent pendant les hivers rigoureux. Avec la semence jarovisée, toutes les plantes montent à graines dans la même année, mais les tiges sont mal ramifiées, et, par suite, le rendement en semences est trop bas,

La jarovisation des choux navets, du chou frisé et du chou moellier est irréalisable, parce que les germes sont insensibles au froid.

PRINTANISATION DES SEMENCES PREALABLEMENT SOUMISES A L'ACTION DES HAUTES TEMPERATURES

par Roger **David**

Faculté des Sciences de Bordeaux

J'ai remarqué, au cours de recherches antérieures, que les hautes températures agissant sur les graines normales (non humidifiées) provoquent, lorsque la durée de chauffage n'est pas trop prolongée, une inhibition provisoire de la germination. J'ai voulu savoir dans quelle mesure un tel chauffage préalable modifie l'accomplissement de la printanisation et s'il pourrait être utilisé pour ralentir la germination pendant ce traitement.

Dans ce but, j'ai recherché, pour diverses espèces, la durée de chauffage à 100°C qui provoque un retard sensible à la germination (sans tuer les graines). A 105°C, une de mes élèves, Mlle J. André, a obtenu des résultats un peu différents. Ces durées sont (exprimées en minutes) à 100°C : Blé, 40 ; Orge, 20 ; Seigle, 15 ; Radis, 5 ; Moutarde blanche, 4 ; et à 105°C : Blé, 20 ; Orge, 10 ; Lupin, 14 ; Vesce, 8.

Des semences ainsi chauffées ont été soumises au traitement de printanisation par le froid (gonflage des graines dans l'eau, puis séjour plus ou moins prolongé à + 2°C). Les graines traitées (chaleur et froid) ont été semées en même temps que deux lots de semences témoins, l'un constitué par des graines seulement printanisées, l'autre par des graines non traitées, mais gonflées pendant 24 heures avant le semis.

La première expérience a été effectuée en 1952, celle de mon élève, Mlle André, en 1953. Voici d'abord les résultats obtenus dans la première.

La durée de printanisation à + 2°C était de 40 jours et le semis a été effectué le 2 avril 1952. Pour les céréales, les témoins sont restés à l'état végétatif. Tous les *Blés* printanisés (semences préalablement traitées ou non par la chaleur) sont montés, ont fleuri et fructifié sensiblement à la même époque. Pour l'*Orge*, deux durées de chauffage préalable ont été essayées : 16 et 20 minutes. Dans le cas de la première, la montée, l'épiaison, la floraison et la fructification ont été simultanées chez les divers sujets printanisés, traités ou non par la chaleur. Une durée de chauffage de 20 minutes a entraîné un retard d'une vingtaine de jours à la montée, la floraison et la fructification. Avec le *Seigle*, je n'ai observé qu'un retard d'une quinzaine de jours à la montée, la floraison, la fructification. Après chauffage et printanisation des graines de *Radis*, les montée, floraison et fructification des sujets printanisés chauffés ou non, ont été à peu près simultanées ; la printanisation a entraîné une avance de 10 jours par rapport aux témoins non traités. En ce qui concerne la *Moutarde*, le chauffage préalable a provoqué une légère avance de 3 jours par rapport aux sujets traités seulement par le froid et une avance de 8 à 10 jours par rapport aux témoins non traités.

Cette première expérience, effectuée en 1952, montre que le traitement préalable par la chaleur n'affecte souvent que d'une manière assez légère le développement de la plante. Toutefois, pour des durées suffisamment prolongées, l'accomplissement de ce phénomène a été partiellement empêché par le traitement préalable par la chaleur.

Pour la deuxième série d'essais (chauffage préalable des semences à 105°C), les semis ont été effectués les 31 mars, 16 avril, 7 mai 1953. Les résultats suivants ont été obtenus :

Blé : pas de montée chez les sujets non printanisés. Semis du 31 mars (45 jours de printanisation), la chaleur (20 m.) retarde la montée de 15 jours et l'épiaison de 8 jours. Semis du 16 avril (61 jours de printanisation) ; retard de 7 jours à la montée et à l'épiaison ; avec seulement 44 jours de printanisation, la chaleur empêche la montée. Semis du 7 mai : 82 jours de printanisation ; la chaleur empêche la montée de la plupart des sujets, retarde d'une dizaine de jours celle des autres.

Orge : semis du 31 mars ; la printanisation avance d'un mois la montée et l'épiaison ; la chaleur diminue cette avance d'une huitaine de jours. Semis du 7 mai : pas de montée pour les sujets non printanisés ; la chaleur retarde la montée et l'épiaison d'une dizaine de jours.

Lupin : semis du 31 mars ; la printanisation (34 jours) provoque une avance de 15 jours à la floraison ; la chaleur la diminue de 6 à 7 jours. Semis du 16 avril ; la printanisation (51 jours) avance la floraison de 15 jours ; la chaleur diminue cette avance de 7 jours.

Vesce : semis du 31 mars ; la printanisation (33 jours) avance la floraison d'une vingtaine de jours ; la chaleur (8 minutes) diminue cette avance de 8 jours. Semis du 16 avril. Printanisation : avance de 20 jours ; la chaleur diminue cette avance de 14 jours. Semis du 7 mai ; printanisation : avance de 7 jours ; la chaleur supprime cette avance.

Cette deuxième série d'expériences montre que le traitement préalable par la chaleur diminue l'avance à la montée, l'épiaison et la floraison lorsque le semis est assez précoce ou le traitement de printanisation suffisamment long ; il supprime complètement cette avance lorsque le semis est assez tardif ou que la durée de printanisation par le froid est plus courte.

En résumé, un chauffage convenablement prolongé des graines entraîne une inhibition partielle de leur germination ; les plantes issues de telles semences, qui subissent en outre un traitement de printanisation, ont un développement toujours accéléré par rapport aux témoins (non traités). mais retardé par rapport aux sujets printanisés dont les semences n'ont pas été préalablement chauffées. Il semble que, pendant cette période, les phénomènes provoqués par l'action des basses températures s'accomplissent plus lentement que pour les graines non chauffées. Tout se passe comme si la réaction de synthèse du précurseur d'une hormone florigène était plus ou moins ralentie. Ces résultats confirment l'opinion de **Chouard** suivant laquelle « les inhibiteurs de croissance inhibent à la fois la germination et le déroulement de la vernalisation ». On ne peut donc utiliser le chauffage préalable des semences pour diminuer la vitesse de germination pendant la printanisation.

L'ACTION DE LA TEMPERATURE SUR LA REPRODUCTION DES VEGETAUX

par Jean **Séchet**
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Au cours de nos recherches sur la printanisation, nous avons été amené à effectuer quelques constatations susceptibles de préciser les interprétations données au sujet de ce phénomène. C'est plus particulièrement le rôle joué par la température, en liaison avec l'élaboration d'une substance favorisant la reproduction, qui sera examiné ici à la lumière de quelques expériences que nous allons résumer.

Un semis de Lupin blanc, effectué le 23 mars 1949, comprenant des lots de graines printanisées pendant 7, 61, et 113 jours (P. 7, P. 61 et P. 113), et un lot de graines non traitées (T.), a permis de noter les dates de floraison suivantes :

Traitement	1ère floraison	2ème floraison	3ème floraison
P. 113	18 Mai (56)	1er Juin (70)	13 Juin (82)
P. 61	18 Mai (56)	8 Juin (77)	22 Juin (91)
P. 7	8 Juin (77)	28 Juin (97)	
T.	13 Juin (82)	28 Juin (97)	

Les nombres figurant entre parenthèses indiquent la durée, en jours, de la période semis-floraison. Ces durées sont les mêmes pour la première floraison de P. 113 et de P. 61 ; pour la seconde floraison P. 113 a 7 jours d'avance sur P. 61 et pour la troisième floraison cette avance est de 9 jours. Le lot P. 7 a 5 jours d'avance sur le lot T. à la première floraison et perd cette avance à la seconde floraison.

Ces faits nous ont conduit à penser que *pendant le traitement, une substance hypothétique favorisant la reproduction serait élaborée en quantité* *proportionnelle à la durée d'exposition aux basses températures. Cette substance serait utilisée lors de la floraison.* En effet, pour la première floraison P. 113 et P. 61 disposent de quantités supérieures à la dose nécessaire ; à la seconde floraison la portion restante ne permet plus l'avance maximum chez P. 61, d'où diminution de l'avance par rapport à P. 113 ; le même phénomène se reproduit pour la troisième floraison. Dans le cas de P. 7, la petite quantité existante permet, à la première floraison, une avance de 5 jours par rapport au témoin (T.) ; la faible dose épuisée, ces deux lots ont alors leur seconde floraison à la même date.

Des plantes de Blé Vilmorin 27 cultivées en serre à température à peu près constante de 15° C peuvent se reproduire convenablement en l'absence de basses températures. Ces mêmes plantes, semées dans les conditions naturelles après le 15 mars, ne parviennent pas à se reproduire, et même pour des semis printaniers moins tardifs les pourcentages de pieds montés et d'épis fécondés demeurent faibles. Une adjonction ou une diminution de lumière ne peut induire la floraison lorsque la plante provient de semis printaniers.

La lumière n'ayant aucun effet, les basses températures n'étant pas indispensables, nous avons pensé que *l'obstacle à la reproduction pouvait provenir des hautes températures que subissaient précocement ces plantes* lorsqu'elles étaient cultivées en plein air.

Des semis de Lupin blanc effectués à une année d'intervalle (7 juillet 1948 et 7 juillet 1949) comprenant chaque fois les mêmes lots de graines traitées (P. 32 et P. 10) et témoins (T.) ont donné les dates de floraison suivantes :

Dates de semis	Traitement	Date de floraison	Durée semis-floraison
7 VII I948	P.32	I7 Août	4I
	P.I0	I5 Sept.	70
	T.	29 Sept.	84
7 VII I949	P.32	25 Août	49 (+ 8)
	P.I0	5 Oct.	90 (+20)
	T.	24 Nov.	I40 (+56)

Pour expliquer les différences d'une année à l'autre, considérons les sommes des températures maxima et minima subies par ces plantes :

Périodes	Somme des températures en 1948		1949	
	Maxima	Minima	Maxima	Minima
7 Juil. - 17 Août	1099,7	610,5	1308,5	657,4
7 Juil. - 15 Sept.	1826,7	1013,5	2179,7	1131,8
7 Juil. - 29 Sept.	2197,6	1173,8	2624,1	1332,9

On constate que pour des périodes équivalentes (durées semis-floraison de 1948), les plantes ont subi en 1949 des températures nettement plus élevées. *L'effet retardant de ces hautes températures est d'autant plus marqué que la plante a subi un traitement plus court.*

Enfin, des semis de Lupin blanc furent effectués le 17 août 1950, après des traitements de 36 et de 53 jours (P. 36 et P. 53) et sans traitement (T.), d'une part sur le terrain d'expériences sous les conditions naturelles de température et de lumière et, d'autre part, en serre, à température variable et sous des durées d'illumination variables (6 h. 30-10 h.-normale-continue). Toutes les plantes printanisées donnent deux floraisons. En serre *ces floraisons sont d'autant plus précoces que la durée quotidienne d'éclairement est plus longue.* Les plantes non traitées fleurissent tardivement sur le terrain mais ne parviennent pas à fleurir en serre et ceci malgré les différentes durées d'éclairement.

Les plantes non traitées du terrain montrent que le froid n'est pas indispensable pour leur reproduction. Pour expliquer l'absence de floraison des plantes de la serre, effectuons un examen comparatif des températures maxima et minima subies par les plantes dans la serre et sur le terrain.

	SERRE		JARDIN	
	Somme des températures			
	Maxima	Minima	Maxima	Minima
du 17 au 31 août	467	284	454	236
mois de Sept.	799	447	761	363
mois d'Oct.	702,5	411,5	655	332
mois de Nov	527	343	448	219

Ces sommes sont toujours plus élevées dans la serre, ce qui *milite en faveur de l'action empêchante des températures élevées.*

En résumé, si nous rapprochons ces résultats, il nous semble possible d'affirmer, d'une part, qu'un précurseur d'une substance de floraison prend naissance pendant le traitement de printanisation, d'autre part, que la floraison est empêchée lorsque la plante subit l'action de températures trop élevées surtout au début de sa croissance. L'élaboration de ce précurseur serait possible à des températures différentes des températures de printanisation, mais une réaction antagoniste le détruirait ou l'utiliserait à d'autres fins à des températures élevées. Il se transformerait ultérieurement, sous l'action de la lumière, en une substance favorisant la floraison.

ESSAI DE PRINTANISATION DES VEGETAUX PAR LA CHALEUR

par R. Schweitzer
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Nous avons entrepris, sous la direction du Professeur David, sur diverses plantes cultivées, des essais de printanisation par la chaleur (25 à 32° C) en vue d'une étude comparative avec les résultats obtenus à l'aide de basses températures.

Une difficulté technique a dû d'abord être vaincue : des graines maintenues à la température de 25° C pendant un mois, avec 75 % d'humidité, moisissent très souvent et germent avec une rapidité telle qu'elles sont bientôt impropres au semis ordinaire et souvent même à la plantation. Nous avons surmonté ce double risque en établissant dans l'étuve de traitement un agitateur à mouvement saccadé, brassant les graines toutes les demi-heures par un brusque mouvement de bascule du bocal qui les contient.

Les espèces botaniques sur lesquelles ont porté nos essais sont les suivantes : Blés d'automne (Capelle, Champagne, Aurèle Gaby et Japhet) ; Blé de printemps (Fylgia) ; Maïs hybride United 22 et 28 ; Pois Vilmorin Alaska ; Vesce de printemps ; Lupin blanc ; Betterave fourragère ; Radis rose, puis diverses plantes ornementales qui ont été abandonnées.

Chaque espèce a fait l'objet de plusieurs lots, ordinairement 3, parfois 6 ou 9, permettant des essais comparatifs : un lot traité au froid (3° C), un autre traité à la chaleur, le troisième non traité servant de témoin. Quand il y avait deux ou trois séries de trois lots, nous faisions varier, soit la température, soit la durée du traitement, soit la date de semis.

Voici, très succinctement, quelques résultats obtenus :

Pois : 23 jours à 28° C. 200 pieds environ. Avance à la floraison de 14 jours sur le témoin ; rendement 175 % de celui du témoin. Pour le lot printanisé au froid (23 jours à 3° C) ces chiffres étaient de 4 jours et de 186 %.

Radis : 15 jours à 25°, 15 jours à 30°. Avance à la tubérisation (date où les racines atteignent 17 mm de diamètre) de 8 jours. A pleine maturité, la moyenne de diamètre des racines était de 29,6 mm pour les sujets printanisés au froid, contre 23,5 mm pour les témoins ; 21,3 pour les sujets à 25° C et 20,9 pour les sujets à 30° C. Par contre, chez ces deux derniers lots, la racine demeure plus longtemps tendre, consommable, et pleine ; l'éclatement intérieur et la lignification se montrent plus précoces chez les témoins.

Blés d'automne et de printemps : résultats peu marqués et contradictoires. Les essais sont abandonnés.

Vesce de printemps : chaleur 28° C pendant 25 jours. Avance à la floraison de 9 jours. Croissance : 1 m 40 de hauteur moyenne contre 1 m 12 au témoin et 1 m 19 au froid. Rendement en graines : 237 % du témoin.

Cotonnier : 17 jours et 27 jours à 30° C. Avance à la floraison de 11 jours pour l'apparition de la première fleur, et 9 jours pour la pleine floraison. Nombre moyen de fleurs par pied : 17, contre 13,6 aux témoins.

Remarque : chez les sujets de cotonnier printanisés par la chaleur, la croissance a été stoppée à 78 cm en moyenne, contre 95,6 chez les témoins. De plus, les fleurs se groupent très sensiblement à la même hauteur sur le végétal, alors qu'elles sont éparses sur les témoins. La fructification n'a pu être atteinte, par suite d'un semis trop tardif, sauf pour quelques sujets qui n'avaient pas de témoins de leur âge.

Lupin blanc : 25 jours à 27° C. Ces sujets se rapprochent beaucoup plus des témoins que ceux du lot printanisés au froid. Ces derniers ont 29 jours d'avance à la floraison, mais ne donnent de fleurs que sur un seul étage (les rameaux secondaires ne fleurissent pas), et le rendement en graines est inférieur. Avance de 5 jours seulement chez les sujets printanisés à la chaleur. Fait assez étonnant : absence presque totale de nodosités sur les racines des sujets traités (froid ou chaleur), présence normale sur les témoins. Mais ce fait appelle encore confirmation.

Ricin et Maïs. Les sujets traités à la chaleur ont été d'une remarquable venue et d'une très bonne fructification. Malheureusement les témoins correspondants ont subi des accidents de végétation interdisant toute comparaison sérieuse. Les essais se poursuivent.

Il est à noter que les graines sortant de l'étuve après 25 à 30 jours de traitement sont ordinairement en assez mauvais état et ne peuvent être semées directement : placées à 15° C entre deux feuilles de papier buvard humide, elles régénèrent leurs racines en 2 ou 3 jours et donnent ensuite une excellente reprise.

INFLUENCE DE LA TEMPERATURE SUR LE TALLAGE EPI DU BLE

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Etudiant le déterminisme physique de la croissance et du développement du blé, l'A. a défini en 1949 (1) une loi d'action spécifique de la température sur le rythme de développement du blé et plus spécialement sur le processus de la *montée* qui débute au moment où les entre-nœuds des différentes tiges commencent à se différencier. Cette loi s'exprime sous la forme d'une courbe présentant un maximum. Elle définit pour chaque valeur de la température un certain coefficient d'efficacité *C* d'autant plus élevé que la température considérée est plus favorable. Les résultats obtenus confirmaient et expliquaient ceux apportés précédemment par la printanisation (vernalisation), montrant notamment que la zone des températures optimums pour un blé d'hiver se situait vers 2° et 3°C.

Par ailleurs, les expériences faites par l'A. en 1943, en refroidissant et en réchauffant directement le sol au voisinage du point végétatif d'un blé semé normalement, tendait à montrer (2) que l'action spécifique de la température devait jouer non pas tant sur le rythme de développement proprement dit que sur le nombre total de tiges susceptibles de *monter* en épi. Or, si l'on examine les résultats des essais de comportement de diverses variétés de blé effectués dans les différentes régions de France, on constate, en ce qui concerne les variétés d'hiver (celles qui réagissent le mieux à la printanisation), en semis d'automne, toutes conditions égales par ailleurs, que le *tallage épi*, cest-à-dire le nombre d'épis par pied à la récolte, est systématiquement plus élevé dans la région Nord de la France ou la région parisienne que dans une région comme la Normandie ou la Bretagne.

En partant des résultats rappelés ci-dessus, ce fait semblait pouvoir s'expliquer en invoquant justement cette action spécifique des températures relativement basses sur la *montée* des variétés d'hiver, les températures moyennes journalières automnales, hivernales et du début du printemps étant dans l'ensemble plus basses dans la région parisienne qu'en Bretagne, par exemple. Pour essayer de vérifier cette hypothèse, il apparaissait possible d'opérer de la manière suivante. Pour la période de végétation intéressée, que l'on a choisie arbitrairement comme correspondant à la période novembre-avril, on peut considérer, au lieu des températures moyennes journalières successives, les coefficients d'efficacité *C* correspondants. En faisant alors la sommation des valeurs successives de *C*, soit ΣC, si l'hypothèse émise est valable, on doit constater que le tallage épi est d'autant plus grand que ΣC est elle-même plus élevée.

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(1) *C. R. Acad. Sci.*, T. 229, 1949, 898-899.
(2) *Thèse*, Paris 1944.

C'est effectivement le résultat auquel l'A. aboutit en considérant deux séries de données culturales différentes : d'une part, des données se rapportant uniquement à Versailles (région parisienne) et correspondant à une dizaine d'années ; d'autre part, des données se rapportant à la fois à Versailles et à Rennes (Bretagne) intéressant les mêmes variétés de blé, en semis d'automne, effectués dans des conditions comparables. Dans les deux cas, les corrélations obtenues peuvent s'exprimer graphiquement par des relations linéaires, les coefficients de régression étant nettement significativement différents de zéro pour $P = 0{,}05$. Sur le plan de la pratique culturale, les résultats précédents soulignent l'un des aspects de l'action favorable d'un hiver froid sur le rendement, aspect non négligeable puisque le tallage épi représente un des facteurs essentiels de ce rendement. Ils permettent aussi d'expliquer, au moins en partie, certaines différences plus ou moins systématiques de rendement observées suivant les régions de culture.

PHYSIOLOGICAL AND CHEMICAL CHANGES OCCURRING PRIOR, DURING AND SUBSEQUENT TO GERMINATION OF SOME ROSACEOUS SEEDS

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Seeds of *Prunus persica, Rhodotypos kerrioides* and other rosaceous species produce abnormal seedlings when forced to germinate without the prechilling treatment. The internodes of these physiologically stunted plants are telescoped and instead of elongating, they increase in girth. This type of growth is similar to that obtained when vegetative buds of the north temperate zone plants are not sufficiently winter-chilled. By grafting, the seat of dormancy in the dwarfs was found to be in the shoot — thus seed dormancy is in a sense bud dormancy. Lack of elongation is not the sole limiting factor in these stunted plants since subjecting the dwarfs to red light causes the internodes to elongate — however, the tip remains dwarfed. In regard to the localization and movement of essential elements, radioactive phosphorus (P^{32}) when applied to the soil was readily transported to the leaves and growing tip of the dwarfs, although not quite as rapidly as in the control plants. Normal seedling development can be obtained by subjecting either the seeds (for 2-3 months) or the dwarfs (for 6-8 weeks) to a temp. of 5°C. The changes occurring at 5°C are accumulative since dormant seeds produce dwarfs, partially after-ripened seeds produce semi-dwarfs, and fully after-ripened seeds produce normal plants. As after-ripening progresses at 5°C, the insoluble reserve materials in the seeds gradually decrease while the soluble materials increase. Other changes also occur such as increases in water absorption power, acidity, activity of various enzymes, etc. In agreement with earlier studies of increases in soluble nitrogen, tests with paper chromatography show that amino acids develop during after-ripening. Experiments in progress are concerned with the identification and rate of formation of these various acids both during and following the period at 5°C and also when seeds are forced to germinate without the low temperature pretreatment. Various chemicals have been applied to the seed, the soil, and introduced directly into the dwarfs but, thus far, no effective substitute for low temperature has been found.

ROOT GROWTH OF *ACER SACCHARINUM* SEEDLINGS DURING WINTER

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There is some difference of opinion in literature over the extent to which root growth of deciduous trees is able to proceed during winter. Some experiments performed in this laboratory suggest a possible reason for this disagreement.

It has already been demonstrated that root elongation of 1st-year seedlings of *Acer saccharinum* L. is closely dependent upon some activity on the part of the leaves (1). Various disbudding and defoliation experiments have since revealed that at the end of the growing season, just after the termination of height growth, this role of the leaves is transferred to the newly formed terminal bud. Soon after the formation of the terminal bud, root growth stops and, under laboratory conditions, at a relatively constant temperature of 20°C ± 2.0°C, no further root growth was observed for a period of at least 6 months. When seedlings were exposed to low temperatures outside the laboratory, however, new root growth was observed after about 3 months. This apparent low temperature requirement for the initiation of root growth obviously suggested a relation with the known low temperature requirement for the completion of dormancy in the terminal bud.

This relation was confirmed by a series of experiments in which dormant seedlings were exposed to differential shoot and root temperatures in the equipment described in (2). It was found, for example, that when seedlings were given a minimum period of 2 months at a shoot temperature of 5°C and a root temperature of 15°C, new root growth ensued. Disbudding, or stem ringing just below the terminal bud, brought about an immediate cessation of root growth. Whenever growing roots were present, raising the shoot temperature to 15°C always resulted in the immediate expansion of the terminal bud ; keeping the shoot at 5°C, however, inhibited but expansion, but did not interrupt the transmission of the root growth stimulus from the bud.

It was argued, therefore, that root growth can only proceed during winter when contact with a non-dormant terminal bud is maintained. Temperatures below the minimum necessary for bud expansion, however, do not necessarily interfere with, or mask, the influence of the bud.

In other experiments, dormant seedlings were given 2 months at 2°C and then exposed to a series of root and shoot temperatures between 2°

and 27°C. It was demonstrated that under our experimental conditions the minimum temperature for the initiation of new root growth, is 5°C. The minimum for bud expansion, however, is between 10° and 15°C.

Thus, under natural conditions in winter, root growth of *Acer saccharinum* seedlings before visible leaf expansion, is possible provided that two conditions have been fulfilled. In the first place, the low temperature requirement for overcoming bud dormancy must have been satisfied. This requirement is equivalent to 2 months at a shoot temperature of below 5°C and is not influenced by root temperature. Secondly, the shoot temperature must be between 5° and 10°C and the root temperature above 5°C.

If this relation between initiation of root growth and non-dormant condition of the terminal bud is a general phenomenon — and not limited to the seedling stage of *Acer saccharinum* — it is clear that failure to take it into account and, further, failure to distinguish between true physiological rest, and dormancy imposed by the environmental conditions, may lead to contradictions about whether or not root growth starts before leaf expansion in the spring.

References. — 1. Richardson (S. D.). *Proc. Kon. Ned. Akad. v. Wetensch.*, Amsterdam C, LVI, 346-353 (1953). — 2. *Ibid.*, 185-193 (1953).

L'ACTION DU FROID SUR LE METABOLISME D'UNE PLANTE DE PLAINE CULTIVEE A HAUTE ALTITUDE

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Parmi les facteurs invoqués pour expliquer la morphologie des plantes alpines, l'alternance des hautes et basses températures a attiré depuis longtemps l'attention. A la suite des résultats déjà obtenus, il nous a paru intéressant de préciser les mécanismes de cette action.

Des expériences ont été faites sur la Betterave demi-sucrière cultivée à haute altitude (2.860 m) en plein vent, et dans un châssis pourvu d'un système de chauffage permettant de soustraire les plantes à l'abaissement nocturne de la température (Culture comparative à basse altitude en plein vent, et dans un châssis soumis à un refroidissement artificiel durant la nuit).

Les individus de haute altitude cultivés en châssis chauffé offrent les caractères des plantes de plaine et présentent une tubérisation presque normale. Les individus de plaine soumis à l'action du froid nocturne rappellent les individus de haute altitude et se caractérisent comme eux par une tubérisation très réduite.

Le dosage des glucides à différents niveaux de l'appareil végétatif révèle

un ralentissement considérable des migrations chez les plantes soumises à l'action du froid nocturne. Ceci peut expliquer l'accumulation des glucides dans les organes aériens et l'absence de tubérisation.

Le dosage des acides organiques fait apparaître, sous l'effet du froid, un enrichissement des feuilles en acide malique et surtout en acide citrique, traduisant une diminution des oxydations et une augmentation des carboxylations favorisées par la succulence des organes.

L'étude des protides indique un ralentissement de la protéogenèse.

Les mesures de pression osmotique révèlent l'existence de deux périodes successives chez la Betterave de plaine : une période de jeunesse (P. O. : 10-15 atm), une période adulte correspondant à la phase de saccharogénie (P. O. : 25-28 atm). Chez les individus de montagne, la pression osmotique s'installe d'emblée à une valeur élevée (25-28 atm).

En conclusion, la physiologie de la plante de haute altitude se caractérise par un ralentissement du métabolisme, de la croissance et de l'évolution morphologique. Le freinage des migrations provoque l'accumulation des produits de la photosynthèse dans les parties aériennes, de telle sorte que la construction des tissus se fait dans des conditions de concentration anormalement élevée. La forme alpine est le résultat d'un nanisme nutritionnel et d'un déséquilibre métabolique.

STORAGE TEMPERATURE AND BULBING OF GARLIC (*ALLIUM SATIVUM* L.)

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The garlic of commerce is grown mainly in mild climates where it is planted in the fall or early spring. The bulbs mature and the tops die down by midsummer. In some regions garlic produces flower-stalks (with topsets but no seed) while in other areas flower-stalks are rarely found.

Previous studies by the author have shown that garlic is a long-day plant with respect to bulbing, and that maturity is hastened by low temperature storage of bulbs prior to planting and by warm growing temperatures in the spring.

Recently an extended study of the effect of pre-planting storage temperatures on plant growth has been made, and several rather distinct and interesting responses have been observed.

The experimental technique consisted of storing mature garlic bulbs at 0°, 5°, 10°, 15° and 20°C for periods of one to several months before planting in the field or greenhouse.

The effect of storage temperature on growth may be summarized as follows :

1. Dormancy. At time of harvest garlic cloves sprout slowly and erratically. After a minimum of 4 to 6 weeks' storage at 10 or 15°C, cloves sprout readily. With increased length of storage cloves stored at 10°C and finally 5°C sprout most quickly. Cloves stored at 20°C never sprout quickly or regularly, regardless of length of storage.
2. Plant growth and yield. Storage of bulbs at 5°C to 15°C prior to planting induces early and rapid growth. However, preliminary data indicate that, except for late spring plantings, yield is more dependent on planting date than on pre-planting storage temperatures.
3. Growth habit. Plants from bulbs stored at 0°C and 5°C have narrow leaves and long false stems. These plants also produce irregular shaped bulbs at maturity. Storage at 0° to 10°C for one or more months prior to planting may make bulbs too rough for good market quality.
4. Time of maturity. For certain planting dates, storage at 0° or 5°C may hasten maturity by as much as one month.
5. Bulb formation. Plants grown in the greenhouse (18°C minimum temp.) from cloves which have not received prolonged low temperature storage prior to planting do not form bulbs even during the long days of summer (lat. 38.5°N).
6. Flower induction. Flower-stalks are uncommon in the area where these tests were carried out and were not produced by low-temperature storage of the bulbs prior to planting. Factors inducing flower-stalk formation are as yet obscure.

3. *PHOTOPERIODISME*

RAPPORT

THE MECHANISM OF PHOTOPERIODISM

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1. *Introduction.*

Photoperiodism is the phenomenon that plants react to the relative length of day and night, of photoperiod and nyctoperiod. There is a wide variety of responses, while different plants may react in a completely different way. The most conspicuous reaction is flowering, and practically all work has been done on this process. Therefore, we shall, with the exception of § 9, confine ourselves to flowering.

The mechanism of photoperiodism is unknown. However, enough experimental facts have come to our knowledge to make the problem open to discussion. The human mind is usually much ahead of actual facts in building up hypotheses on the real nature of a process and this holds true for photoperiodism. It seems the only way to make progress, but we should be aware of the fact that we may have to abandon our concept, if further experimental data necessitate this. Such is the general background of this paper.

We can only deal with some of the more important facts on the photoperiodic action in so far as, in our opinion, they may have something to do with the mechanism. We emphasize that photoperiodism is but one group of factors in the physiology of flowering and that a better way to put the problem would be : What part does photoperiodism play among the circumstances which cause a plant to flower ?

Several more extensive reviews of the subject have been written : Gregory (1947), Hamner (1947), Melchers and Lang (1948), Murneek and Whyte (1948), Leopold (1951). The most recent one (1952) is the comprehensive survey of Lang (1) (*), to which we refer for all of the literature not specifically mentioned here.

2. *Developmental phases in flower formation.*

As « juvenile phase » with regard to photoperiodism we define a period from sowing in which the plant does not yet react to the relative

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(*) These figures refer to Literature Citations at the end.

length of day and night. In literature several indications, such as « ageing » and « a minimum vegetative size » point to this juvenile phase. Its existance has perhaps been established best of all in *Campanula medium*, where relatively young plants do not react to short day, while beyond a certain age the response increases rapidly to a maximum (Wellensiek, 2). Probably « ripeness to flower » or « puberty » marks the end of the juvenile phase.

We next distinguish between the invisible induction (a preparative action) and the visible realization, again to be divided in flower bud formation, elongation, flowering proper, etc. Most work on photoperiodism has been concentrated on induction and the first steps of realization. It is important to draw a sharp line between these two (see § 3). It may well be that the juvenile phase for realization lasts longer than that for induction, while also a juvenile phase for induction may be absent, but since in most cases induction and realization seem to be favoured by the same set of circumstances, it will be difficult to separate the two.

3. *Types of reaction.*

Thus far, plants have been divided into four classes with regard to their photoperiodical responses, namely *indifferent* (*indeterminate, day neutral*) *plants, long day plants* (L.D.P.), *short day plants* (S.D.P.) and *intermediate plants,* which may be regarded as a special group of S.D.P. (1). Two more classes must be added ; their characteristics are as follows.

In « *short-long day plants* » flower formation takes place when a period of short days is followed by a period of long days. Only one case is known, namely *Campanula medium* (Wellensiek, 3).

In « *long-short day plants* » flower formation takes place when a period of long days is followed by a period of short days. The only case known is *Bryophyllum verticillatum* (Dostal, 4).

Whether or not completely indifferent plants occur remains to be seen. Most of them react to some extent to differences in day length, in such a way that, on the whole, they approach L.D.P. The short-long day and long-short day plants can be better understood if we assume that induction and realization are favoured by different circumstances. In short-long day plants the induction is then favoured by short day, the realization by long day, while long-short day plants react in just the opposite manner. With regard to the first step in flowering, namely the induction, these plants may then be looked upon as S.D.P. and L.D.P., respectively.

This conception leaves us with only L.D.P. and S.D.P., with a wide range of quantitative differences within each group. The most extreme cases, viz. plants that « never » flower unless the photoperiods are long or short, have been indicated as « qualitative » L.D.P. and S.D.P., respectively. However, closer study reveals that the reaction is always « quantitative ». **Lang** and **Melchers** have demonstrated that the L.D.P. *Hyoscyamus* can flower under short day conditions provided the temperature is relatively low. They think that the disappearance of the inhibitory action of the dark period in L.D.P. at low temperature is quite general. **De Zeeuw** (5) could make *Perilla,* hitherto considered a typical qualitative S.D.P., flower in L.D. and even in continuous light by lowering the light intensity.

Hence the sharp line between L.D.P. and S.D.P. disappears and a « qualitative » photoperiodical action no longer exists.

4. *Light intensity.*

The products of photosynthesis are essential for flowering : When enough storage material is present or when sugar is supplied, many plants have been shown to flower in complete darkness. When photosynthetically active light is given, a short daily period is often sufficient, but L.D.P. will not flower unless this is supplemented with light which may be of even such a low intensity that accumulation of photosynthetical products cannot occur. In S.D.P. low intensity light, supplementing the day length until it exceeds the critical, inhibits flowering.

If a long nyctoperiod is interrupted by brief exposures to light, this may lead to flowering in L.D.P. and to the suppression of flowering in S.D.P., both in short day conditions. For the action of this interrupting light the product of intensity and duration evidently is the determining factor. As far as can be judged from the data in the literature, the possibility of a photosynthetical action of interrupting light is not excluded, but this is not the generally accepted conception.

5. *Action spectra.*

To establish the action spectra of photoperiodically active light three approaches are being made. **Van der Veen** (6) and **Wassink** and **Stolwijk** (7) study light given during the whole photoperiod, **Wassink** et al. (8) study the supplementary light, while **Borthwick**, **Hendricks** and **Parker** (9) are studying the interrupting light. Most progress has been made in the last case. Red light is most active, while a second much smaller maximum occurs in the blue part of the spectrum. The red light effect is completely and repeatedly reversible by infrared radiation. The percepting pigment is regarded as an open chain tetrapyrrol related to phycocyanin and the photoreaction as a cis-trans isomerisation (**Hendricks**, 10).

A similar action spectrum was found for supplementary light, except in some Cruciferous plants, where the development of flowers is strongly promoted by blue, violet and infrared. The action spectrum of light given during the whole photoperiod is surprisingly enough the opposite of that of supplementary light, at least as far as elongation is concerned ; the effect on flowering has not yet been studied.

6. *The function of the leaves.*

The perception of light takes place in the leaves. Hence the leaves perceive the photoperiodical action. One leaf may be sufficient for photoinduction. Completely defoliated plants are insensitive to photoperiodism. Leaves in an unfavourable photoperiod exercise an inhibiting action (*Kalanchoe*, HARDER, 11, *Poinsettia*, VAN DER VEEN, 12). Leaves do not exert an inhibiting influence in continuous darkness (L.D.P. *Spinacia*, S.D.P. *Xanthium*). Two cases are known, the L.D.P. *Hyoscyamus* and the S.D.P. *Chenopodium amaranticolor*, that flower in complete darkness when defoliated. A recent case is the L.D.P. *Beta* (FIFE and PRICE, 13).

Photosynthesis, which is an essential prerequisite for flowering, takes place in the leaves. It appears, however, that in addition to this stimulative action the leaves under unfavourable photoperiodical circumstances also exert an inhibiting influence on flowering, because defoliation may lead to flowering. DE ZEEUW (14) has demonstrated that also in plants, considered as indifferent, the young leaves may retard flowering. It would then seem plausible that the essence of photoperiodism is the removal of the inhibiting action of the leaves.

7. *Transportation of the product of photoperiodism.*

It has frequently been supposed that the product of photoperiodism is a positive stimulus to flower which can be transported from the leaves to the growing tips. Certain results of grafting experiments have been considered as proof for this concept. LANG (1, p. 270) has tabulated these cases. Several have never been published, while some have not been supported by other workers in experiments with other or even with the same plants. However, it seems beyond doubt that flowering can be obtained under unfavourable photoperiodical circumstances when the grafting partner is in a favourable photoperiod. However, no flower formation takes place unless the receptor has been defoliated. Consequently, referring to the effect of defoliation as discussed above, it is plausible that by defoliation an inhibition is removed, while flower bud formation becomes possible, because products of photosynthesis are transported from the leaves of the unhibited donor.

Although not related to photoperiodism it may be mentioned that DOORENBOS (15) clearly demonstrated that generative *Hedera helix* as a result of grafting upon vegetative *H. helix* reversed to the vegetative condition, whether or not stock and/or scion were defoliated.

8. *Growth hormones.*

Growth hormones definitely have something to do with flowering (see e. g. BONNER and BANDURSKI, 16 ; LEOPOLD, 17). Several observations point to an antagonism between growth and flowering. From this it would follow that growth promoting substances have a tendency to retard or inhibit flowering, be it directly or indirectly. It might be that in S.D.P. the action is more direct than in L.D.P.

With the possible exception of *Ananas sativus* artificial application of growth hormones inhibited flower formation in all S.D.P. plants tested. The anti-auxin triiodobenzoic acid (TIBA) did not exert any direct influence on S.D.P., perhaps with the exception of *Xanthium* (BONNER, 18).

In L.D.P. an important influence of the temperature was demonstrated by LEOPOLD and GUERNSEY (19). Application of growth promoting substances favoured flowering at relatively low temperature, but inhibited flowering at high temperature.

9. *Other responses to the photoperiod.*

It seems reasonable to suppose that all processes sensitive to the photoperiod are closely related with regard to their mechanism. We confine ourselves to some phenomena which point to the importance of growth promoting or inhibiting substances.

Growth of certain perennials depends on day length. In several cases short day induces winterdormancy. e. g. *Fragaria* (VAN DEN MUYZENBERG, 20) and *Populus* (VAN DER VEEN, 21). HEMBERG (22, 23) has presented evidence that winter-dormancy is caused — or at least accompanied — by elongation inhibiting substances. In other cases the physiological changes induced by day length are reversible, e. g. in *Rhododendron catawbiense*, where the plants stay dormant in photoperiods shorter than 12 hours, but resume growth soon after the day length is increased. In continuous light only terminal shoots develop (unless the temperature is very high), in shorter photoperiods mainly lateral ones (DOORENBOS, unpublished).

In several cases tuber formation is favoured by short day. With *Helianthus tuberosus* VAN DE SANDE BAKHUYZEN and WITTENROOD (24) could increase the length of the stolons in S.D. conditions by application of growth substances, while they could shorten the internodes in L.D. by applying triiodobenzoic acid.

10. *The mechanism of photoperiodism.*

The main trend of the foregoing discussion is that a plant will flower, if it has passed its juvenile phase, if necessary has been vernalized and contains an adequate amount of food substances, provided the leaf does not exert an inhibiting action. If this is so, photoperiodism is the removal of this inhibition. It is unnecessary to assume the synthesis of a flower-forming hormone as a direct result of photoperiodism and it is much more evident not to do so. Whether or not a « florigen » or « anthocaline » exists at all is beyond the scope of the present discussion. We emphasize that we only deny the formation of a flower-forming substance as an immediate outcome of the photoperiodic action.

To supplement the evidence presented in the foregoing, we may cite the following data, which support our conception. WYCHERLEY (25) has shown that the product of vernalization in *Cynosurus cristatus* is immobile. If there is a flower-forming hormone, it is synthesized *in the growing tip* under the influence of long day. Since in this synthesis evidently both the product of vernalization and a « long day substance » are involved, it is clear that the latter as such cannot be the flowering hormone.

Coming now to the mechanism of the photoperiodic action, we must first discuss the schemes that have been proposed thus far. In the following summary of the most important schemes we have made some slight changes in the symbols to render comparison more easy. P means precursor ; A and B are undefined conditions or substances ; FFS is flower-forming substance ; LFS is leaf-forming substance.

HAMNER, for S.D.P. :

$$\begin{tikzcd}
\text{light} + CO_2 \arrow[r] & A \arrow[dr] & \\
& & FFS \\
\text{in darkness } P \arrow[r] & B \arrow[ur] &
\end{tikzcd}$$

HARDER and BODE have proposed a scheme which is essentially the same, except that they postulate a substance Z, produced by photosynthesis that protects B — which they call X — against photodestruction.

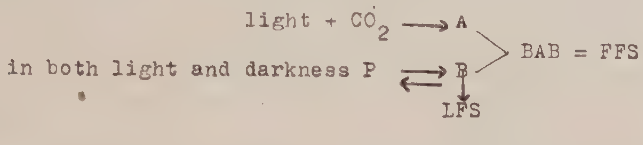
GREGORY, for S.D.P. :

$$\begin{tikzcd}
& LFS & & \\
\text{light} + CO_2 \arrow[r] & A \arrow[u] \arrow[r, shift left, "\text{darkness}"] & B \arrow[l, shift left, "\text{light}"] \arrow[r] & FFS
\end{tikzcd}$$

GREGORY, for L.D.P. :

$$\begin{tikzcd}
& LFS & \\
\text{light} + CO_2 \arrow[r] & A \arrow[u] \arrow[r] & FFS
\end{tikzcd}$$

Van de Sande Bakhuyzen (26) for both L.D.P. and S.D.P. :



These schemes have in common : (1) a substance A, formed in strong light when CO_2 is present, and (2) a light sensitive substance B. In Gregory's S.D.P. — scheme, B is formed from A. Hamner and Van de Sande Bakhuyzen both suggest that A and B in some way interact to form the FFS.

Van de Sande Bakhuyzen carries this further in postulating that A and B combine to form either AB, in which B may still be destroyed by light, or the light-stable BAB which is the FFS. He further suggests that in L.D.P. the rate of production of B, symbolized as b, is equal or more than equal to twice the rate of production of A, symbolized as a, while in S.D.P. b/a lies between 1.0 and 2.0. The explanation of the effect of a long night on S.D.P. then is that the concentration of A is lowered until BAB can be formed instead of the light-sensitive AB, while in L.D.P. during a long night the supply of A is soon exhausted, causing the concentrations of B to rise until it reaches the threshold value for the production of the LFS.

Of course we agree that products of photosynthesis (A) are necessary for flower formation, but we do not find sufficient evidence for a flower-forming substance. This leads to the conception that A and B are not chemical substances, reacting to form another substance that is the FFS, but rather that AB or BAB represent certain balances between the products of photosynthesis and « B ». In our line of thought « production of B » in the schemes mentioned may equally well represent « disappearance of an inhibitory influence ».

Two main problems emerge from the above discussion. The first is : *What is the nature of the inhibition ?* In view of the work done on the influence of auxin on flowering (see sub 8, and also von Denffer (27)), it seems quite reasonable to suppose that in S.D.P. the auxin level in a long day is too high for flowering, while the dark process consists of the removal of auxin. For L.D.P. a tentative working-hypothesis has been advanced by Laibach and Kribben (28) that the auxin level in a daylength below the critical is too low for flowering, while long photoperiods are necessary to raise it.

The schemes discussed may be brought into line with this conception, if one reads for B « removal of auxin » and instead of BAB or FFS « *a certain balance between the products of photosynthesis and auxin* ».

The second main problem is how the favourable photoperiod removes the inhibitory action of the leaves. This question can now be put as :

How does the photoperiod affect the relation between the products of photosynthesis and auxin? For completeness' sake we mention that long and strong light increases the amount of photosynthetical products. However, the amount of information on the influence of the photoperiod on the auxin level is as yet very small. It seems certain that in general the concentration of auxin in the leaves increases during the photoperiod and decreases during the nyctoperiod, see e. g. LAIBACH (29) and BECKER (30). Auxin may be inactived by light in the presence of carotin or riboflavin, see e. g. REINERT (31), but this offers no explanation of the effect of supplementary or interrupting light, as none of the action spectra as discussed sub 5 agrees with those of carotin or riboflavin. On the other hand, no evidence has been presented to show how the action spectra established by PARKER *et al.* and WASSINK *et al.* affect the auxin level.

We look forward to further revealing work along this line, as well as in all approaches to photoperiodism. In many respects only the surface of the problem has been touched so far.

11. *Conclusion.*

From a brief discussion of the main experimental results it is concluded that no specific flower forming hormone arises as a direct result of photoperiodism. As a working hypothesis it is suggested that flower formation is determined by a certain balance between products of photosynthesis and auxin. Leaves in unfavourable photoperiods inhibit flowering through an unfavourable relative auxin level. Photoperiodism removes this inhibition by bringing the balance between photosynthetical products and auxin to a value which enables the plant to form flowers.

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ON PLANTS GROWN EXCLUSIVELY IN LIGHT OF RESTRICTED SPECTRAL REGIONS

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The effects of « coloured » light on various life processes of plants have since long been extensively studied. During the last years more refined studies have been made in various fields. Most of the studies available have either studied selective effects of regions very restricted with respect to wave length on small areas in low or high intensities during short periods, or more general effects of a limited number of broad spectral regions in the growth and development of plants. We have especially aimed at studying the effects of various narrow regions of high spectral quality, in high light intensities over large areas and during long periods on the growth, the photoperiodic behaviour, and the formative reactions of plants. It was intended to study the whole photobiologically active spectral range in great detail, especially including the near infrared. Better aspects for realizing this aim have arisen in recent years owing to the introduction of new light sources, especially fluorescent tubes with selectively emitting fluorescent compounds. By combining such tubes with special glass filters light of high intensity in various restricted spectral regions has been obtained (see (1)). First an equipment was built up in which narrow regions, covering violet, blue, green, yellow, red, and near infrared were obtained on area's of ca. 2 × 0.5 m, in intensities up to 3000 ergs/cm^2/sec. (1). It was found that the intensities available were sufficiently high for the growth of certain algae, but not for that of higher plants. The equipment was used for studying the effects of daylength extension with coloured light in plants receiving a short day in high intensities of artificial white light under controlled conditions. Some of Funke's results were confirmed and, additionally, it was found that the near infrared showed photoperiodic and stem-elongating effects resembling those obtained under similar conditions with blue light. J. A. J. Stolwijk will report on some of the findings with this equipment before this Congress. Next we developed and equipment consisting of glass cabinets, 110 × 30 cm wide and 70 cm high, mounted at 3 sides with light sources and glass filters (2). In these cabinets plants are in a more or less uniformly illuminated space ; all cabinets are adjusted at the same energy-intensity. This amounts to about 14000 ergs/cm^2/sec., but in this case we prefer evaluating the energy as influx into a space which is feasible when measuring with a spherical radiation meter which yields ergs/sec./cm² cross section of sphere (see (3)). The intensities in the various cabinets expressed in this unit are about 56000. We have these cabinets in violet (400 mμ), green (540 mμ), yellow (590 mμ) and red (660 mμ), and we recently added blue (460 mμ) and infrared. In the latter, the infrared radiation (from incandescent lamps) has been supplemented by an amount of (photosynthesizable) red light allowing plants to develop. Also a cabinet with white light (from fluorescent tubes) is added as a general reference. The temperature is mostly kept at 20° C during the light period (mostly 16 h. p. day) and 15-17° during the dark period. A number of plants have been investigated so far, work with others is being started. Good growth is obtained in all cabinets with various species. So far, they agree in showing normal shape in the white light, and in the violet in which they remain even shorter than in the white light. In green and especially in yellow and red light they show strong elongation of stem internodes and in some cases, of leaves. With blue and infrared so far experience was only gained with the tomato. The shape in blue is much the same as in white ; there is somewhat more elongation than in violet. The development in the infrared was less well than in red, notwithstanding the equal amount of photosynthesizable radiation. The internodes are strongly elongated, and the leaves are small. In yellow and red light, the tomato shows a strong elongation also of the midribs of the leaves; which, as well as the leaflets, moreover show a strong, epinastic curvature. The whole reminds of an excess of « auxin-action » as compared with blue and white light. This is now being investigated by J. A. J. STOLWIJK.

Arachis hypogaea, cultivated in the white, violet, green, yellow, and red cabinets showed no (or very slight) opening of the leaves in yellow and red light, a normal day-light position in white, and somewhat less in green, whereas the day-light position was « exaggerated » in violet (4). The effects of blue and infrared in this respect have not yet been examined under comparable conditions.

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THE PHYSIOLOGICAL LENGTH OF THE PHOTOPERIOD

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Since the fundamental work done by *Garner et Allard* it was generally thought that the photoperiodic action on plants depended solely on the number of light hours which the plants receive daily.

Later on, I discovered that the sensitivity of different plant species was by far not the same. Strawberries and *Kalanchoe* f. i. need about a ten times higher intensity for daylengthening than Chinese *Aster* and *Poinsettia*. Therefore an intensity of incandescent light of 1 Lux (6 mW/m2 visible radiation), given to Strawberries during 8 hours nightly, acted like darkness, that is to say : not at all (Roodenburg, 1939).

So it is rather difficult to determine the active length of a natural day, as a certain day of the year will be longer for *Poinsettia* f. i. than for *Kalanchoe*. The astronomical day length is well defined, but the part of the twilight which is photoperiodically active is not a constant one.

Therefore I thought it necessary to speak of a physiological daylength (Roodenburg, 1947) which varies for different plant species and with the brightness of the sky. It is not easy to determine these physiological daylengths, as experiments with very low intensities of daylight are rather difficult to realize. For this purpose we need some kind of artificial daylight. In this respect, there is another factor of importance : namely the spectral composition of the light. It was soon found that in many plant species red light was more effective than blue light, when used as a supplement of daylight. So, weak mercury light often fails to have any photoperiodic effect, owing to its lack of red rays. On the other hand we got good results with the red Neon light. A much stronger photoperiodic effect however, was obtained with incandescent light, the radiation of which having its maximum in the near infra red (Roodenburg, 1940).

A disadvantage of the use of ordinary incandescent lamps for promoting plant growth, was generally the abnormal lengthening of the stems. However, the stretching of internodes is a well-known phenomenon in plants under long day conditions. During the course of my experiments it became more and more clear to me that the lengthening influence of incandescent light was due to an excessive photoperiodic action of its radiation.

This light source has a much more daylengthening effect than daylight itself, owing to the excess of the short wave infra-red rays of the tungsten filament lamp (Roodenburg, 1952).

Regarding this, it must be possible to obtain quite normal plants in very short daily periods of incandescent light. Irradiation with incandescent lamps during winter, given only at daytime, could be applied without any harmful effect (tomato).

I found that day length does not only influence seedlings in a ripe — to — flower stage, but also during the early development following germination (f. i. *Kalanchoe*). In this period many seedlings, including those of dayneutral plant species, show under long day conditions an apparent elongation and under short day conditions short internodes and petioles. The short day conditions prove to have a retarding effect on the growth of stems ; the more lengthening effect a particular light source has, the more the duration of the light period must be shortened to obtain a sturdy plant.

Therefore it was possible to obtain a short day effect with long photoperiods of blue light only, in a dark cabinet (V. D. VEEN (1950).

It is my opinion that, owing to its lack of red rays, this blue light treatment acted here like natural short days. So, it does not ensue that equal photoperiods of light of different spectral composition work out the same physiological day length. When incandescent light is used for growing plants in dark rooms, we have to choose short daily periods of irradiation, shorter than the natural photoperiods.

In this connection the results obtained by research workers in the field of preventing etiolation by light are of interest. TRUMPF (1924) showed that short daily light treatments are sufficient to prevent abnormal stem lengthening and the etiolated habit of plants in darkness. When giving one hour light a day we found that this effect can be obtained with all sorts of artificial light sources. We tried Neon, mercury, fluorescent and incandescent light of medium intensity. All were equally effective ! So, with short daily irradiations, it is possible to obtain short stems in incandescent light too. Thus the light period of short days serves a. o. to prevent etiolation.

Elongation of stems occurs as well in darkness as in extreme long days. It also occurs in long periods of red light in which blue violet rays are lacking. On the contrary stems remain short under short day conditions, and also in longer periods of blue light without red rays.

Summarizing we may say : the physiological length of a photoperiod is highly dependent on the spectral composition of the sort of light used.

The physiological daylength increases in the sequence : mercury, fluorescent, Sodium, Neon and incandescent light. It is likely that for most plant species, the photoperiodic action of sunlight lies between that of fluorescent and that of sodium light.

ANTAGONISTIC EFFECTS OF SUPPLEMENTARY LIGHT OF VARIOUS WAVE LENGTH REGIONS

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It has been shown in experiments under controlled conditions of temperature and light intensity that plants exposed to a short day in white light, supplemented by low intensities of light of various spectral regions respond differently to different wave length regions. Most photoperiodically sensitive plants show a strong photoperiodic response to supplementary light in the green, yellow and red regions of the spectrum, and little or no response to the violet, blue and infrared regions. Cruciferous plants, however, react strongly in the blue and near infrared and show little or no response to green, yellow and red light. Apart from the photoperiodic effect, strong formative effects also result from supplementary irradiation with light of various spectral regions (1, 2, 3). Elongation of stem, petioles and leaves occurs upon supplementary irradiation with blue and infrared wave length regions. Some of the evidence obtained with *Brassica Rapa* f. *oleifera* subf. *annua,* made probable that the middle regions of the spectrum (520-700 mμ) had an effect on elongation of internodes antagonistic to that of the regions 450-500 and 700-900 mμ. This indication was obtained from a comparison of two treatments, *viz.,* 8 hours strong white light per day + 4 hours white light of low intensity, and 5 hours strong white light per day with 4 hours blue supplementary light. The plants receiving 8 + 4 hours white light remained short, whereas the plants receiving 4 hours blue light after a basic illumination of 5 hours in strong white light elongated strongly, notwithstanding the fact that during the additional irradiation the latter received only about one third of the energy in the blue region that the former received after the first five hours. This led to the conclusion that the remainder of the white light had an antagonistic effect. It may be mentioned that Parker *et al.* after our first reports on antagonism of various spectral regions discovered obviously similar effects with respect to light sensitive seed germination.

It was established that supplementary irradiation during one hour per night at an intensity of 1000 ergs/cm^2/sec was nearly as effective in producing elongation of *Cosmos* internodes as the usual exposition of 8 hours per night at the same intensity. The intensity of supplementary light required for maximal response to blue and infrared wave length regions under our conditions was found to be about 400 ergs/cm^2/sec of blue supplementary light, and about 250 ergs/cm^2/sec in the infrared. The spectral regions blue and infrared on the one hand, against green, yellow and red on the other hand were tested for antagonism in two ways.

Supplementary light was given consisting of two spectral regions applied either consecutively or simultaneously, *e. g.* in *Cosmos,* in different proportions. The results of both types of experiments clearly indicate that the action of the wave length regions 450-500 mμ and 700-900 mμ, visible as elongation of *Cosmos* internodes to a greater length than that reached in the dark controls, disappears when supplementary light of wave length regions from 520-700 mμ is added, either simultaneously or subsequently. When antagonistic wave length regions are given consecutively, the effect of the last applied spectral region persists. In the case that two spectral regions are given simultaneously the effect depends on the proportions of the two in the mixture.

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A REVERSIBLE PHOTOREACTION CONTROLLING PHOTOPERIODIC RESPONSE, SEED GERMINATION AND OTHER PHENOMENA

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Photoperiodic control of flowering of *Xanthium saccharatum,* a short-day plant, and the light regulation of germination of seed of *Lactuca sativa* var. Grand Rapids have identical action spectra. These action spectra have two very striking features, one a region in the red that inhibits flowering of *Xanthium* and promotes germination of *Lactuca* seed and the other a region in the far red having the exact opposite effects of promoting flowering of *Xanthium* and inhibiting germination of *Lactuca* seed.

The red region of high effectiveness extends from about 6000 to 6800 A, with a maximum near 6500 A. Effectiveness decreases with wavelengths shorter than 6000 A and longer than 6800 A. Radiation in the farred part of the spectrum that reverses the effects of red is very active from about 7000 to 7600 A, with a maximum near 7300 A. Activity decreases sharply at wavelengths shorter than 7000 A and longer than 7600 A.

Reversible effects of red and far red on flowering of *Xanthium* are readily demonstrated by applying the respective kinds of radiation as successive interruptions near the middle of each of three daily 12-hour dark periods. If the dark periods are not interrupted the plant flowers, but if radiation of about 6500 A is given briefly near the middle of each dark period it does not flower. The stimulus to flower can be restored in such red-irradiated plants by radiation of about 7300 A immediately after they receive the red.

Inhibition of flowering by red and its repromotion by far red can be effected by less than 1 minute of each type of radiation. Such inhibition-promotion cycles have been used four times, sample lots of plants being returned to darkness after each of the eight irradiation periods. All plants returned to darkness after red failed to flower and all returned to dark after far red flowered.

Successive irradiations with red and far red of light-sensitive seeds of *Lactuca* reverse the germination response exactly the same as identical treatments reverse the flowering response of *Xanthium*. For instance, seeds are held in darkness on moist blotters for 1 to 4 hours and are then subjected alternately to red and far red for 1 to 10 minutes. These durations of red and of far red depend on degrees of promotion and inhibition desired, the intensities of red and far-red light used and other conditions of the experiments. Samples are returned to darkness at the end of each of the successive treatments with red and far red. Lots returned to darkness following red show the same degree of promotion of germination regardless of the cycle in which red was last given and those returned to darkness following far-red treatment are equally inhibited regardless of cycle. Experiments involving five such cycles have been performed with seeds of *Lactuca sativa* and involving eight cycles with seeds of *Lepidium virginicum*. The same type of reversibility of germination has also been demonstrated with seeds of *Lepidium densiflorum, Nicotiana tabacum* and *Ulmus americana*.

Reversibility and identity of action spectra for flowering response of *Xanthium* and germination response of *Lactuca* to red and far-red light clearly show that the two phenomena are controlled by identical photochemical reactions. The spectral region that is inhibitory to flowering of *Xanthium* is exactly the region that is promotive of germination of *Lactuca*. The far-red wavelength region that is promotive of flowering is also the precise region that is inhibitory to germination.

This reversible photochemical reaction that controls photoperiodic response and seed germination appears to be of universal occurrence among plants. Effects of the reaction, moreover, are expressed in many responses that appear superficially unrelated to photoperiodism or germination. Among these is control of production of a yellow pigment in the cuticle of fruits of some varieties of *Lycopersicon esculentum*. This yellow pigment is present in cuticles of fruits ripened in light but is absent in fruits ripened in dark. Formation of the pigment in dark-ripened fruits can be induced by brief daily irradiations with light of very low energies during the 5- to 10-day period of ripening. The action spectrum for formation of the yellow pigment is the same as that for inhibition of flowering of *Xanthium* and for promotion of seed germination of *Lactuca*. Far-red radiation of about 7300 A following red radiation of about 6500 A results in colorless cuticles. The effects of red followed by far red thus cancel leaving the apparent effect of uninterrupted darkness. If fruits that have received one cycle of red and far red are immediately given additional cycles they respond to the final treatment with red or far red the same as they would have responded to the first had treatment been stopped at that point.

This same reversibility is exhibited by the long-day plants *Hyoscyamus niger* and *Hordeum vulgare*, red radiation being promotive and far red inhibitory to flowering. Growth of various structures of dark-grown seedlings is also regulated by the same reversible reaction. Red radiation inhibits elongation of the second internode of *Hordeum vulgare* and the hypocotyl of *Phaseolus vulgaris* and promotes expansion of the primary leaves, elongation of the petioles and disappearance of the plumular hook of *P. vulgaris*. Far red reverses each of these responses to red.

TIME DEPENDENCIES IN PHOTOPERIODISM

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Growth phenomena controlled by the reversible photoreaction described in this symposium depend in three general ways upon time. These ways are (1) the time constant of the reversible reaction ; (2) the time for interaction of the active form of the pigment with other reactants leading toward control of development ; and (3) the time course of development such as germination, floral initiation, floral maturation, leaf and stem growth, pigmentation of bracts and fruits, bulb formation, etc.

In darkness the far-red-absorbing form (7300 A maximum) of the effective pigment changes slowly to the red-absorbing form (6500 A maximum). The half time for this thermal reaction as measured at 35°C by germination response of *Lactuca sativa* seeds is of the order of 24 hours. The reaction is photoreversible in either direction as rapidly (seconds) as was permitted by the radiant energy supplied. The rate of the photoreaction is temperature independent as well as time independent.

The radiant energy of appropriate wavelength required for promotion or inhibition of germination of *L. sativa* or *Lepidium virginicum* seeds by a fixed amount varies with the time of water imbibition. The promotion and inhibition sensitivities are coupled in such a way that when the one increases the other decreases. *L. sativa* seed increases in sensitivity to promotion for the first 12 hours of water imbibition at 20°C, remains constant for about 8 hours, and then decreases. The sensitivity to inhibition at first decreases, then remains constant, and finally increases. At 20° *L. virginicum* seed becomes constant in sensitivity after about 24 hours. The changes in relative sensitivity in the two directions can be as great as 100 fold.

L. sativa seeds promoted in germination by red radiation can be completely suppressed by far-red radiation after intervals as great as 4 hours at 20°C. With longer time intervals between two irradiations the germination response is less reversible and after 12 hours is not reversible. In other words, after a time interval of some hours of darkness the germination process if promoted has passed beyond the point of inhibition by radiation. The germination, of course, is best observed only after several days.

Floral initiation of *Xanthium saccharatum* if inhibited by red radiation as an interruption of a dark period adequate for initiation can be reversed (promoted) by far-red radiation provided the time interval is less than 30 minutes. With longer time intervals between irradiations the initiation is not reversible. If far-red irradiation (greater than 7000 A) is given for 1 minute after red irradiation initiation is reversed, but if it is continued for 30 minutes initiation is not reversed. Both forms of the effective pigment are absorbing in the region between 7000 and 7500 A, but in this region the absorption coefficient for the far-red form is the greater of the two. Consequently both forms of the pigment are continuously excited. The observed response indicates that the excited pigment is reacting with another molecule leading toward control of development.

In *X. saccharatum*, a short-day or rather a long-night plant, the excited combined form of the effective pigment inhibits floral initiation and development. The pigment is maintained in this form during illumination, as during the day. In darkness this combined form reverts to either the red- or the far-red-absorbing form of the pigment. The rate of this reaction and the time required for the biological response that it inhibits to pass adequately beyond the point of control together afford a measure of time in photoperiodism.

THE INFLUENCE OF PHOTOPERIOD ON CO_2 METABOLISM IN KALANCHOE

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A dwarf variety of *Kalanchoe Blossfeldiana* (a plant requiring about 15 short days to flower), was placed with its upper part in a translucent gas-tight chamber. Air was circulated through the chamber and its CO_2 content followed by infra-red absorption. The plants and chambers were in a temperature-controlled room with artificial light. In this way the uptake and evolution of CO_2 by the plant could be followed in light and in dark for an indefinite period. With the plants on long days, photosynthesis occurs normally, and in the dark period there is a slight net CO_2 uptake, as would be expected for a succulent plant. On short days, however, the pattern changes markedly. After about 10 short days (8 hours' light) the CO_2 uptake in the dark increases markedly, and in the light period the onset of photosynthesis is followed by a rapid evolution of CO_2 which lasts several hours. The extent of these processes increases with increasing numbers of short days, until after 33 short days the total metabolism in the light periods shows a net production of CO_2, while that in the dark period shows a net uptake.

The CO_2 fixation comes to an end in the dark after 12 to 16 hours, however long the dark period.

If the 16-hour dark period is interrupted at its midpoint by 10 minutes of full light, the progressive change of metabolism is largely prevented, and the plants behave nearly like those on long-day. This holds up to at least 25 such interrupted dark periods.

If instead of placing in the light, the plants are transferred (still in the chambers) to 30° in the dark, CO_2 is evolved. On subsequent illumination a further small evolution of CO_2 occurs. There are therefore both thermolabile and photolabile CO_2-yielding constituents. High temperature during the dark period also reduces the flowering response to short days.

The results can be explained as follows : successive long dark periods permit the gradual formation of an enzyme system which fixes CO_2 in the dark. To do this, a CO_2 acceptor is required, and this is formed from photosynthetic products. The cessation of CO_2 fixation after 16 hours or darkness may be due to exhaustion of this acceptor, or may be ascribed to translocation out of the leaves either of the acceptor or of the enzyme system. The product of CO_2 fixation is decomposed by light or high temperature, or both. It is moderately stable in the dark at 25°. The enzyme system is destroyed by light during the first part of the dark period but subsequently becomes photostable. These phenomena strongly indicate a close relationship between CO_2 metabolism and flowering.

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* The experiments were begun with the collaboration of Prof. F. G. Gregory at Imperial College, London. They were continued and completed at Harvard University.

TRACER STUDIES IN THE BIOCHEMICAL ASPECTS OF FLOWERING

by S. P. **Sen** and A. C. **Leopold**

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Recent investigations in this laboratory have indicated that carbon dioxide fixation in the dark period plays an important role in the flowering of short day plants. The products of CO_2 fixation by three photoperiod-sensitive plants *Xanthium*, Biloxi Soybean and Wintex Barley were studied by subjecting the plants to different light and dark conditions in an atmosphere of $C^{14}O_2$.

A large amount of the $C^{14}O_2$ fixed in the dark period appears in the fraction insoluble in 80 % ethanol. The rate of $C^{14}O_2$ — fixation into the soluble fraction depens on the nature of the plant and the induction treatment. Long dark periods have a retarding effect on the rate of photosynthesis which follows.

Chromatography and radioautography of the soluble fraction demonstrated that $C^{14}O_2$ is metabolised into a large variety of compounds. The products formed in successive induction cycles are essentially similar but important differences were observed between the labeled metabolites of an inductive and non-inductive treatment. Most of these involve the Krebs cycle acids and phosphorylated compounds.

Some labled compounds which may possibly be related with flowering have been traced to the reproductive buds of *Xanthium* and the implications of the findings are discussed.

ADAPTIVE ENZYMES, ENDOGENOUS RHYTHMS AND THE PHOTOPERIODIC BEHAVIOR OF PLANTS

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It has recently become clear, mainly through the researches of Bünning, that there exists an intimate relationship between the endogenous rhythms in plants and the kind of light regime they require for the initiation of flowers. For instance, five varieties of soybeans which have well-marked rhythmicity in the sleep movements of their leaves are definite short-day plants, while five other varieties which have no such rhythmicity in sleep movements are day neutral. It therefore seems likely that no explanation of the mechanism of photoperiodism will be satisfactory which does not simultaneously explain the nature of these endogenous rhythms. This communication will present a model system for producing endogenous rhythms in plants, and will further show the applicability of the proposed mechanism to an explanation of photoperiodism.

The theory rests upon the following facts :

(1) All of the endogenous rhythms known to plant physiologists, such as the sleep movements of leaves and flowers and root pressure, can be explained by rhythmic fluctuations in the concentration of auxin.

(2) The native auxin of plant tissues is either indoleacetic acid (IAA) or some close chemical relative, such as indoleacetonitrile or ethylindoleacetate.

(3) The IAA in plant cells is in a dynamic state, being constantly synthesized from precursors such as tryptophan, and constantly destroyed by the enzyme complex known as IAA oxidase. Both the rate of synthesis and the rate of destruction are affected by light.

(4) IAA oxidase is an adaptive enzyme, being inductively formed in response to auxin levels of about 10^{-7}M, with a lag period of about 30 minutes.

The theory, based on these facts, is as follows : IAA is produced in the meristematic areas of the plant and, in the absence of IAA oxidase, rises in concentration in the cells. When the concentration of IAA rises to about 10^{-7}M, IAA oxidase formation is induced, but maximal oxidase activity does not appear until several hours later. When it appears, it rapidly oxidizes IAA, recreasing its level in the cell. Once the level of IAA has fallen, the level of the adaptive IAA oxidase in the cell also falls. The synthetic capacity of the cell for IAA will thus again exceed its destructive capacity, and the IAA level will rise. This combination of a synthetic system and an adaptive destroying system are sufficient to produce a constantly oscillating level of IAA.

The effect of illumination on the flowering behavior of the plant will depend on whether the light falls during a period of net synthesis or net destruction of IAA. Since auxin is known to enhance or to inhibit flowering depending on its level in the cell, we have here a mechanism by which light can control flowering indirectly through its effect on auxin level, which in turn probably acts by affecting the production of the floral hormone.

SUR LE CHIMISME PROPRE A L'ACCOMPLISSEMENT DE LA PHOTOPHASE ET SUR L'EXISTENCE D'EXTRAITS FAVORISANT LA FORMATION DES FLEURS

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I. Chez le Fraisier des Quatre-Saisons à fruits rouges, on peut mesurer l'avancement du développement vers la floraison en utilisant un critère morphologique comme la forme des feuilles, reflet des changements qui affectent le méristème en rapport avec le développement.

Le cours du développement comporte une suite de phases. L'une de ces phases se situe dans la période qui précède immédiatement la floraison. La poursuite du développement y exige des jours longs pendant un certain temps : c'est une photophase. Durant la photophase, la photopériode convenable doit nécessairement s'accompagner de variations déterminées de la température (jours chauds, nuits froides).

L'accomplissement de la photophase aboutit à la floraison.

II. Quelques caractéristiques du chimisme de la photophase sont très remarquables, principalement le métabolisme de la chlorophylle.

Quelle que soit la feuille envisagée, le pigment est chaque jour successivement accumulé, puis dégradé. En jours courts, ou à 20°C constants, — conditions défavorables au développement —, l'amplitude du mouvement est faible et l'accumulation commence dès le lever du jour, tandis qu'en jours longs et en T. variable (jours chauds, nuits froides), — conditions favorables au développement —, l'amplitude est grande et l'accumulation commence 3 à 5 heures après le lever du jour.

En jours longs et T. variable, dans la photophase, la teneur en pigment augmente de feuille à feuille, ce qui correspond à une capacité accumulatrice de plus en plus grande au fur et à mesure qu'on s'approche de la floraison. Ensuite, avec la floraison, le taux de chlorophylle baisse brusquement ; il y a déplacement brutal de l'équilibre vers les activités de dégradation. En jours courts, ou à 20°C constants, l'augmentation de teneur de limbe à limbe est ralentie, et, tandis que la floraison n'a pas lieu, il n'y a pas de déplacement vers la dégradation.

Il existe donc une relation entre les conditions du milieu, le type de métabolisme chlorophyllien, et l'accomplissement normal de la photophase.

III. Une fois sexuée, la plante fleurit pendant plusieurs années. Cependant, la forme des hampes florales varie ; ainsi, en automne, les bourgeons axillaires des hampes ont l'aspect végétatif. Il s'agit, non d'un retour à la période végétative du développement (la plante reste sexuée !), mais d'une déficience locale, temporaire, affectant les bourgeons axillaires.

On peut provoquer ce phénomène de déficience en appliquant des jours courts, ou en se servant des plants croissant le long des stolons. Lorsque ces plants sont reliés par le filet à leur plante-mère en fleur, ils fleurissent, mais si on les isole très jeunes en coupant le filet, la floraison avorte. Les plants isolés, ou les plantes en fleurs transférées en jours courts, conservent le métabolisme chlorophyllien de l'état sexué, caractérisé par la dominance relative de la dégradation du pigment ; mais les deux processus de dégradation et d'accumulation diminuent parallèlement d'intensité. Cette circonstance est liée à la déficience de la floraison.

IV. La relation entre métabolisme chlorophyllien et accomplissement de la photophase jusqu'à la floraison suggère l'étude de l'activité physiologique de produits ayant des liens chimiques avec la chlorophylle, par exemple certains constituants de l'insaponifiable.

La saponification de limbes adultes montre qu'en 8 h., durant la photophase, les caroténoïdes cristallisent dans les cellules, tandis qu'en 16 h., ils restent dissous à l'état de gouttelettes jaunes. Après la floraison, la saponification donne toujours des gouttelettes, très grosses en 16 h., plus petites si on transfère en 8 h.

L'insaponifiable brut est un mélange complexe. L'extrait de plantes en 16 h. est beaucoup plus riche en vitamine E et en stérols que l'extrait en 8 h. ; ce dernier est plus riche en hydrocarbures divers.

En appliquant l'extrait insaponifiable, nous avons réussi à faire fleurir des plants isolés de plantes-mères en fleur (plant-test). Chez les témoins, le bourgeon axillaire de la hampe donne des feuilles ; chez les traités, une fleur. Le plant-test montre que l'activité de l'insaponifiable d'une plante en fleur varie avec les saisons (meilleurs résultats en juin-juillet). Cependant, l'extrait le plus actif n'agit plus si le plant-test est en jours courts : les feuilles en jours courts inhibent son activité. En outre, l'extrait ne provoque pas la floraison, soit de plantules, soit de plantes à la photophase de leur développement.

V. Pour qu'il y ait floraison, il faut que le stade de développement, résultant de l'accomplissement d'une suite de phases préalables, et les conditions de milieu qui y correspondent, concourent à l'établissement et au maintien d'un métabolisme — (en ce qui concerne la chlorophylle : amples mouvements consécutifs d'accumulation et de dégradation, avec dominance relative de la dégradation) —, tel que certains produits soient activement formés dans les limbes — (dont, sans doute, un ou plusieurs éléments de l'insaponifiable) —, et soient utilisables dans les méristèmes, y stimulant la formation des fleurs, comme conséquence de l'activation des divisions cellulaires dans la zone centrale du corps.

THE ROLE OF AUXIN IN THE PHOTOPERIODIC RESPONSE OF LONG-DAY PLANTS

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In the long-day plant *Hyoscyamus niger* (annual variety) floral initiation was induced by treatment with indoleacetic acid. The plants were given a basic eight-hour period of light in the greenhouse plus 18 hours of darkness or of low-intensity supplementary light of various intensities, between approximately 0,2 and 0.01 foot-candles. The auxin was applied daily by spraying the leaves before the beginning of the dark or the low-intensity light period.

Some reports in the literature indicate that auxins may affect flower formation in long-day plants in a quantitative manner, but the experiments reported here appear to be the first in which a qualitative difference in flowering has been observed in response to applied auxin and the significance of auxin in the photoperiodic induction of long-day plants has been demonstrated.

It was found that application of auxin does not induce floral initiation in strict short-day conditions (no supplementary light), but only if the plants are given small amounts of supplementary light during the « dark » period, even though these light quantities are not sufficient to induce floral initiation in the controls (that is, in plants receiving the supplementary light, but no auxin). It was furthermore found that there exists a reciprocal interrelation between the amount of the low-intensity supplementary light which is given to the plants, and the amount of auxin needed to obtain maximal response. With 34 foot-candle minutes, 3 mg/ltr. of indoleacetic acid produced 100 per cent floral initiation ; with 16 foot-candle minutes, the maximal response (40 % of flowering plants) was obtained with 10 mg/ltr. The controls were vegetative in either case.

These results indicate that, although light and auxin are to some extent interchangeable in the photoperiodic induction of long-day plants, auxin does not replace light and *vice versa*, but that light increases the sensitivity of plant to applied auxin. This is in line with the results of recent studies on the action of light and of auxin in the growth of the Avena coleoptile (J. L. LIVERMAN and J. BONNER, 1953). According to these results, light catalyzes the formation of a substance with which the free auxin combines to form an auxin-acceptor complex ; this complex seems to be the form in which auxin exerts its effects on growth as well as floral initiation.

These findings will be briefly discussed with regard to the role of light and of auxin in the photoperiodic response of long-day and short-day plants.

FLOWERING HORMONE OF SHORT-DAY AND LONG-DAY PLANTS

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It has been found that the photoperiodic induction of plants in which flowering is controlled by daylength causes the formation of a flowering stimulus (hormone) in the leaves. This stimulus can pass graft union and induce flowering in a plant which has not been exposed to the inductive daylength conditions.

The graft partner which is under inductive conditions and which is supposed to supply the floral stimulus is called the « donor ». The partner which is under non-inductive conditions and which fails to flower by itself is called the « receptor ».

Hyoscyamus niger (annual strain) or *Nicotiana silvestris* (long-day plants) were grafted on a short-day plant, *Nicotiana tabacum* var. Maryland Mammoth. The induced *Nicotiana silvestris* donors caused Maryland Mammoth receptores to flower. The non-induced partner used as a control did not flower. Similar results were obtained when *Hyocyamus* scions were used as donors. The receptor flowered only with *Hyoscyamus* induced donors. The receptors of the control, *Hyoscyamus* on short-day and Maryland Mammoth on long-day, did not flower.

Maryland Mammoth tobacco induced donors did not cause *Hyoscyamus* nor *Nicotiana silvestris* scions to flower even five months after the grafts were made.

The expriments reported here establish clearly one fact, not known before ; namely, that long day donors induce flowering in short day receptors only, if the former are induced to flower formation themselves.

EFFECT OF INDOLEACETIC ACID AND TRIIODOBENZOIC ACID TREATMENTS ON THE INDUCTION OF FLOWERING IN (1) SHORT DAY PLANTS : (a) *CORCHORUS CAPSULARIS* L., (b) *C. OLITORIUS* L., (c) *SORGHUM ROXBURGHII* VAR. *HIANS* STAPF, AND (2) DAY NEUTRAL PLANT : *BRASSICA JUNCEA* HOOK F. AND THOMS. VAR. P 72

by J. C. Sen Gupta and P. Guha Thakurta

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1. Plants of four species — two species of jute (I) *Corchorus capsularis* L. and (II) *C. olitorius L.,* (III) jowar — *Sorghum Roxburghii* var. *hians* Stapf. and (IV) mustard — *Brassica juncea* Hook. f. and Thoms. var. *P.* 72 were treated with hormones Indole-acetic acid and Triiodobenzoic acid. The first three are short day plants and the fourth a day-neutral plant.
2. Plants kept under normal condition and treated photoperiodically at 10 hrs. (and also 14 hrs. in mustard only) were subjected to treatment of hormones IAA and TIBA, the concentration varying between 5 p.p.m. to 200 p.p.m. in jute, 10 p.p.m. to 20 p.p.m. in jowar, 20 p.p.m. to 40 p.p.m. in mustard.
3. Mode of application of hormones was as spray, applied to soil and dipping in jute and only spray and soil in the other two species.
4. Plants of jute were sown on four dates as follows :
    (1) 15.3.52 at which the plants of *C. olitorius* flower very early — normal plants only.
    (2) 16.4.52 (a) Continuous 10 hrs treatment and normal plants.
    (b) 10 hrs treatment commencing at the age of 30 days and normal plants.
    (3) 2.6.52 *treated* 10 hrs and normal.
    (4) 15.2.53 *olitorius* only which is known to flower very early when sown on about the date — normal.
5. Jowar — sown on 15.6.52. 10 hrs. treated and normal plants.
6. Mustard — sown on 15-10-52 treated at 10 hrs, 14 hrs and normal plants.
7. Except in a very few cases in some of the experiments with jute where some responses are obtained, treatments with the hormones were found to have no well marked effect on the vegetative growth and the initiation of flowering in any of the plants.

UBER BLUHHEMMSTOFFE IN VERKEHRTER TAGESLANGE BEI KALANCHOE BLOSSFELDIANA

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In früheren Versuchen hatten wir festgestellt, dass die Blütenbildung bei der Kurztagpflanze *Kalanchoe Blossfeldiana* negativ beeinflusst wird, wenn *während* oder *nach* der Blüteninduktion Langtag auf die Blätter der Pflanze einwirkt. Im Sommer 1953 haben wir einem grossen Versuch (585 Pflanzen) angesetzt, um zu sehen, wie die Blütenbildung beeinflusst wird, wenn der Kurztagexposition eine Langtagexposition von verschiedener täglicher Dauer voraufgeht.

Die Pflanzen wurden zur Blütenbildung 9stündigem Kurztag ausgesetzt, und zwar während 2, 3, 4 und 8 : Tagen. *Vor* der Kurztagbehandlung bekamen sie während 4 Wochen Langtag, und zwar in 7 Abstufungen, nämlich 12, 12 1/2, 13, 14, 16, 20 oder 24 Stunden. Nach Beendigung der Kurztagbehandlung kamen sie in 13stündigen Tag, also in eine Tageslänge, die für unser Versuchsobjekt nur wenig oberhalb der kritischen Tageslänge liegt. Durch die Langtagvorbehandlung wurde das *Sichtbarwerden der Blüten* umso mehr *verzögert*, je länger der Tag war. Die Verspätung betrug zwischen 8 und 12 Tagen. Noch viel auffälliger war der Einfluss auf die *Anzahl der Blüten* je Pflanze. Bei dauernder Einwirkung 9stündigen Kurztags bildet eine Kalanchoepflanze 500 bis 600 Blüten, bei Vorbelichtung mit 13stündigem Langtag im Mittel nur 142, nach Vorbehandlung mit 14stündigem nur noch 40 und im Anschluss an voraufgegangene Behandlung mit 16- und mehrstündigem Tag entsteht weniger als eine Blüte je Pflanze. So lagen die Verhältnisse bei schwacher Blühinduktion. Bei stärkerer Reizung (grössere Anzahl von Kurztagen) war die Reduktion in der Blütenzahl zwar absolut geringer, relativ war sie aber auch um so stärker, je länger der Tag vor der Kurztagbehandlung war. Auch ohne Zählung der Blüten waren diese Effekte völling eindeutig zu erkennen am Grad der Verlaubung der Brakteen. Bei Vorbehandlung mit relativ geringer Tageslänge waren die Brakteen völlig normal, in den durch den Langtag gehemmten armblütigen Infloreszenzen waren sie umso stärker verlaubt, je länger der Tag vor der Induktion war. Wurden die Pflanzen *vor der Kurztagbehandlung verdunkelt*, so trat eine *Förderung* des Blühens ein.

Die Blütenbildung bei *Kalanchoe* hängt also nicht nur von der Kurztaginduktion ab, sondern auch die Tageslänge *vor* der Kurztagbehandlung ist wichtig. Es müssen sich also im Langtag schon *vor* der Kurztaginduktion Stoffe bilden, die der späteren Blütenbildung entgegenarbeiten. Die hemmende Wirkung des Langtags auf die Blütenbildung beruht also nicht (oder nicht nur) auf einer zerstörenden Wirkung von *bereits vorhandenem* « Blühhormon », sondern auch wenn noch gar kein Blühhormon existiert, bilden sich auch bereits Hemmstoffe, die dann nach erfolgtem Blühimpuls negativ auf das Blühen wirken. Ob es sich dabei um die gleichen Stoffe handelt, die die Hemmung des Blühens bewirken, wenn man *Während* oder *nach* der Kurztagbehandlung Langtag einwirken lässt, erscheint zweifelhaft. Denn diese Stoffe hemmen nach unseren früheren Versuchen immer nur dann, wenn ihre Bildungsstätte sich auf dem Wege *zwischen* dem den Kurztagimpuls aussendenden Blatt und dem Vegetationspunkt befindet ; ihr Wirksamwerden hat also zur Voraussetzung, dass bereits « Blühhormon » vorhanden ist, was bei unseren jetzigen Beobachtungen ja nicht der Fall ist.

Summary : Flower inhibiting substances in non inductive daylength in Kalanchoe Blossfeldiana.

With *K. Blossfeldiana* the effect of short-day treatment on the formation of flowers is diminished if the plants are exposed to a long day before their exposition to a short day. The larger the number of daily light hours, the stronger the retaining effect on the formation of flowers. If the plants are shut off from light previous to the short-day treatment, their flowering is increased. That means that in the plant, when exposed to a length of day which is not favourable to the formation of flowers, substances are produced which retain flowering, independent of the existence of a flower inducing hormone.

RAPPORT

THERMOPERIODICITY AND PHOTOPERIODISM

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For several reasons photoperiodism has become one of the really important problems in Botany. In the first place is the control over day length easily achieved with dark covers or artificial light and thus it is a convenient tool in research. More important from a theoretical point of view is that photoperiodism gives us a tool to study a morphogenetic process : the transformation of a vegetative growing point into a flower primordium.

Under many conditions temperature affects flowering in a manner similar to that of photoperiod. Two different aspects of thermoperiodicity will be discussed. In the first place we should know the extent to which temperature produces the same transformation and changes as photoperiodism, and in the second place, we want to know to what extent temperature and light interact in these responses, and whether one can be substituted for the other.

Before going into details it should be pointed out that photoperiodism is much more general a phenomenon than just the effect of length of day on flowering, for there are also effects on stem elongation, leaf growth and chlorophyll content, etc. The theory of Bünning has related periodic behavior of plants to a general basic phenomenon in the life of a plant, an autonomous rhythm which has a 24 hour cycle. This conception of rhythm takes in such periodic phenomena as nyctinastic movement of leaves, and thus gives photoperiod a much larger role in the growth of a plant as a whole. Bünning's theory claims that over a 24 hour period, plants pass through both a photophil and a scotophil phase. During the scotophil phase light inhibits those physiological processes which it stimulates in the photophil phase. This view does not give a causal explanation of these periodic phenomena, but it provides an excellent description of what is going on, and relates a number of otherwise unconnected phenomena.

Let us start with the consideration of flowering. In general we can say that a large proportion of annual plants from the temperate regions are photoperiodically sensitive, and that their flowering is largely controlled by length of day. Whereas the majority of annuals from colder climates where the growing period is short seem to be short-day plants, most annuals from regions with a winter rainy season and dry summer are long-day plants, and in this way are able to complete their life cycle before the drought period has progressed too far. *Glycine, Xanthium, Chrysanthemum, Callistephus, Cosmos* and *Zinnia* are examples of short-day plants, whereas *Baeria, Hordeum* and *Spinacia* are long-day plants. Tropical annuals seem to be day-neutral and flower specifically at a 12 hour photoperiod. Annuals from northern regions such as *Papaver nudicaule* are long-day plants, perhaps because they grow only under-long day conditions.

When we turn to the biennials we find that although many of them are also long-day plants, practically all of them have, at the same time, special temperature requirements to be fulfilled before they will flower. In beets, *Hyoscyamus* and celery, the plants have to pass through a period of low temperature before they will flower under long-day conditions. It is interesting to note that in both beets and *Hyoscyamus*, genetic races are known which have lost their chilling requirement, and have become ordinary long-day annuals. From an evolutionary standpoint it therefore seems as if the chilling requirement is a more recently acquired characteristic, which can be independent of photoperiodism.

Among perennial plants relatively few cases of photoperiodism are known, but most of them seem to respond to temperature in their flowering response. Not much is known about the relationship of temperature and photoperiod in shrubs, but the following examples show that in some cases such a relationship does exist. In *Camellia,* the initiation of the flower buds takes place only at high temperatures and is independent of length of day. The opening of these flower buds requires low temperatures. This does not mean that the *Camellia* plant is insensitive to day length, for the growth of vegetative buds requires long days, but as far as flowering is concerned, day length does not control this process.

In the peach the opening of the flower buds requires a chilling period of sufficient duration ; therefore, peaches do not flower when kept continuously in a warm greenhouse. Flower initiation occurs in nature about 10 months before actual blooming, and is not influenced by photoperiodism. It was recently found in the Earhart Laboratory by B. Rogers that the initiation of flowers in the peach requires another chilling period, and thus, peaches cannot flower unless they have gone through two successive chilling periods, both of 10°C or lower. Between these, there has to be a warmer period, and long day treatments are effective in keeping vegetative buds in continuous growth. Both short days and low temperatures will cause the buds to become dormant. Thus we find also in the peach that photoperiod affects only vegetative growth.

We have now seen how temperature treatments gradually supplant photoperiodic treatment in their effectiveness in flower induction as we progress from annual to perennial plants. Perhaps this should be stated the other way around ; the emergence of photoperiodism from the thermoperiodic regulation of flowering seems to be connected with the development of the annual behavior from the basic perennial development of plants. The way in which this concept is stated is largely dependent on the evolutionary trend in the growing habits of plants.

Bulbous plants offer a special case in connection with thermoperiodicity and photoperiodism. These have been investigated in great detail by Blaauw and collaborators who found that flower initiation is largely controlled by temperature. The most completely analysed case is that of *Iris Xiphium* var. *Imperator* (Blaauw, 1941). Bulbs of less than 5 cm. in circumference will never flower under any set of conditions and are said to be in a subflorigene state. To reach so called « ripeness-to-flower », a term coined by Klebs, the bulbs must have a circumference of more than 5 cm. After bulbs have reached this size they have attained the preflorigene phase and can be induced to flower by temperatures below 17°C (a 3-months period at 9°C for example, leads to flower initiation). In principle all bulbs behave in the same way. The ripeness-to-flower of tulips and hyacinths also requires a minimum bulb size (7 cm. circumference) but high temperatures cause flower initiation in this case. This ripeness-to-flower does not seem to be a matter of flower-forming substances which must accumulate in the bulbs, and which are not present in sufficient quantities in small bulbs to make them flower. Blaauw pointed out that little bulblets which were in direct contact with a big flowering bulb, never flowered. If the flowering were due to a concentration of flower-forming substances, both small and big bulbs in the same cluster would have bloomed.

The chilling requirement of a number of perennial plants for subsequent flowering initiation, very much resembles the chilling requirement of seedlings of annual plants which is called vernalization. In vernalization it is believed that low temperature causes the formation of special substances which will cause the growing point to be transformed into a flower primordium after they have been transported there. This is based on experiments carried out with embryos detached from, or still in contact with the endosperm. In beets and *Hyoscyamus* however, it was shown that the chilling requirement has to be received by the growing point itself. This followed from experiments carried out by Coville, and later by Zimmerman, with branches of deciduous trees. If individual buds on the branches were given chilling or gas treatment, these particular buds would grow, whereas the non-treated buds on the same branch remained dormant. This would seem to militate against the co-operation of a hormone-like substance under the influence of chilling.

Fifteen or 20 years ago it was pointed out by several investigators such as Thompson, Roberts and Struckmeyer, that the photoperiodic response was modified by the temperature under which it took place. For instance a *Poinsettia* was a short-day plant at low temperatures, but indeterminate at higher temperatures. This brought thermoperiodicity in close association with photoperiodism, since they were interacting instead of substituting for each other, as in the examples mentioned earlier. Not too much work was carried out along these lines because of the difficulty of growing plants at controlled temperatures. The construction of the Earhart Plant Research Laboratory makes it possible to study the inter-action between light and temperature in the flowering of plants in greater detail. Although not much has been published on this, a few examples will be given to indicate the possibilities of this approach.

The strawberry is generally considered as a short-day plant. In numerous greenhouse experiments it was found that short days were essential for the initiation of inflorescences. On this basis, Van den MUYZENBERG worked out a scheme by which he could produce strawberry fruits throughout the year by shortening the day length and using cooling and heating at appropriate times. This short-day behavior could be confirmed for Marshall and other strawberry varieties in the Earhart Plant Research Laboratory. It was found, however, that plants grown at 10°C in continuous light, continue to flower for an indefinite period. This suggested that at low temperatures the strawberry did not require short days. A definite transition between the strictly short-day behavior at 17°, 20° and 23°C, and the indeterminate behavior at 10° and 6°C was found to occur at 14°C, the temperature which Marshall strawberries will initiate inflorescences in both 8-hour and 12-hour photoperiods, but not in 16-hour or 24-hour photoperiods. Above this temperature, no plants receiving 12 hours light per day will flower ; in an 8-hour photoperiod, they flowered abundantly. At both 6 and 10°C inflorescences were initiated and developed in 8, 12, 16 and 24-hour photoperiods.

It was of interest to find what physiological change underlies this shift in photoperiodic behavior. To this end strawberry plants were grown for 8 hours daily in full daylight in a greenhouse at 23°C. During the other 16 hours they were subjected to artificial light at different intensities and different temperatures. It was found that at 20°C inflorescence initiation could be prevented with 200 ft. c. supplemental light, but at 17°C, 200 ft. c. was not sufficient to suppress flowering ; the critical intensity in this case was 400 ft. c. At 14°, flowering was suppressed by light intensities over 700 ft. c., and at 10°, flowering occured even under supplementary light intensities of 1000 ft. c. or more. Therefore we can consider the strawberry a short-day plant at all temperatures, but that the intensity of the supplementary light needed to suppress inflorescence initiation is so high at 10°C or below, that it is not reached either in the laboratory or in nature, and consequently the plant seems indeterminate.

A further insight in the processes causing inflorescence initiation in the Marshall strawberry is brought by experiments in which the number of short-day cycles necessary for initiation at different temperatures was determined. It was found that at 17°C, 8 cycles were required, at 20° and

14°, 11 cycles or more, and at 23° and 10°, 17 cycles. We can conclude that there seem to be two antagonistic processes involved. One process leads to initiation of inflorescences, probably by production of florigen. This process occurs faster at higher temperatures, which accounts for the decrease in number of short day cycles for initiation when the temperature rises from 10° to 17°. Beyond that the increase in necessary short day cycles must be accounted for by a process which counteracts the formation or action of florigen, or causes its destruction. This process has a higer Q_{10} than the other.

The existence of antagonistic processes with high Q_{10} values is known in other cases of flower initiation where light does not play a direct role. Purvis and Gregory have shown that in vernalization, an optimal temperature somewhat above freezing is a compromise between vernalization and de-vernalization processes. In the sugar beet, Stout showed that thermal induction of flowering occurred faster at higher temperatures, but above 10° was counteracted by a process with a high Q_{10}. To translate the thermal induction into flower initiation long photoperiods are essential in the sugar beet. Thus it seems that thermal induction is primary, and photoperiods received later on have to be in the right range to cause the actual inflorescence formation. This is the reverse of the strawberry, where photoperiodic induction is primary, and is modified by temperature. Melchers and Lang found essentially the same behavior in *Hyoscyamus* as Stout had described for the sugar beet. In both plants genetic races are known which do not require the chilling period for flowering, and therefore have become long-day annuals.

Chouard has investigated a very large number of plants as to their photoperiodic behavior, and has found that all possible combinations of strong, slight or no vernalization requirements are found in connection with different types of photoperiodic behavior. In *Matthiola incana* a strong interaction between photoperiod and temperature exists. When plants are grown at temperatures over 20°C, flowering will not occur regardless of whether this temperature is given in the light or dark phase. Yet *Matthiola* plants grow well at 26°C, although they remain indefinitely in a rosette stage. When *Matthiola* plants are grown in continuous light, they will flower at a constant temperature of 20° but in an 8-hour photoperiod they initiate inflorescences only when the night temperatures are 16° or lower. Thus also in this case we find a limited substitution of temperature for light. A similar behavior is found in *Bellis perennis* which can grow and flower at higher temperatures when the photoperiod is longer.

In the preceeding discussion a large number of plants have been discussed in which flowering is controlled either by temperature or by photoperiod or by both. In a few cases it was shown that different processes within the same plant were either photoperiodic or thermoperiodic. We should now point out some vegetative reactions of plants to photoperiod and temperature.

Tomato plants are strictly indeterminate as far as flowering is concerned. However, the vegetative growth is highly sensitive to length of photoperiod. In continuous light of high intensity, the growth soon stops. Dr. Eric BONDE is now investigating the effects of cycles other than 24 hours on their growth. Shortening or lengthening of the 24-hour cycle decreases growth and development very much as one would expect on the basis of BÜNNING's innate 24-hour cycle, but completely different results are obtained when the cycles are shortened to 2-hours at which condition growth is practically normal. The significance of this type of research lies largely in the fact that a physiological analysis of vegetative growth is so much easier than an analysis of flowering. Tomato plants respond very strongly to differences in temperature from day to night. Their vegetative growth and fruiting is strongly influenced by proper thermoperiodic treatments. Recently Mrs. BENDIX has been investigating the effects of temperature fluctuations per se on the growth of tomatoes. When tomato plants were subjected to different temperatures during either the light or the dark period, she found that when the temperature differences did not exceed 6°C, the reaction was the average of the reactions to the 2 different temperatures. With temperature fluctuations exceeding 10°C the plants averaged the temperature when given in the light period, but their growth was strongly inhibited if the temperature fluctuation occurred in darkness.

The subject of thermoperiodicity as well as photoperiodism is not anywhere near exhausted, in fact the recent inclusion of vegetative response has opened up a whole new aspect of this problem. It is also believed that the consideration of both temperature and light effects will help in the theoretical evaluation of growth and development.

EXPERIMENTAL RESEARCHES ON PHOTOPERIODISM
ANALYSIS OF THE MECHANISM OF THE DARK PERIOD REACTION BY THE HIGH-TEMPERATURE INTERRUPTION

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1. The seedling of the Japanese morning glory, *Pharbitis Nil,* known as one of the most sensitive short-day plants was used as a material. At first, optimum nyctotemperature and maximum one for flower formation were determined, and then the phenomenon of the high-temperature interruption in the dark period reaction was observed, moreover analysis of the mechanism of the dark period reaction were carried out by the high-temperature interruption. The inhibition rate was calculated by the general formula which is as follows :

$$H = \frac{V - V_G}{V}$$

where H are the inhibition rate, V the velocity of the dark period reaction (number of flower buds, number of plants with flower buds, number of terminal flower buds, and dry weight per plant) in control, and V_G the velocity of the dark period reaction when it received high-temperature interruption.

2. It seems that optimum nyctotemperature and maximum one for flower formation are respectively about 26° C and 31° C in three cycles of the photoinductive treatment, but in five or seven cycles of the photoinductive treatment flower initiation occurs even at 32° C.

3. When high-temperature interruption (that is 36° C) is given for 1, 2, 4, 6, 8 and 16 hours at the midpoint of the 16-hour dark period, the interruption effects are observed even in the lot of 1 hour and then the inhibition rate increases as the duration of the high-temperature interruption is increased, and it becomes to 100 % even in the lot of 8-hours duration.

4. With the variation of duration of high-temperature interruption (that is, 34° C or 36° C) such as 8, 6, 4, and 2 hours the question was if there was any relationship between the time of the interruption and flower formation (number of flower bud) under three cycles of 8-hour light and 16-hour dark. The high-temperature interruption was arranged to occur at various periods of time ranging from 0 to 16 hours after the treatment of the dark period began. In all the lots, the time effects were observed ;

in general, flower formation was greatly inhibited at the second half of the dark period, especially in the lot of 2-hour duration the time effects clearly seen, that is, maximum inhibition rate occured between 12th and 14th hour of the dark period.

5. All the plants have the terminal flower bud in three cycles of the photoinductive treatment at optimum nyctotemperature. Not all of plants are induced to form terminal floral bud in 8- or 6-hour duration of the high-temperature interruption, in 4- or 2-hour duration the plants in some lots have the terminal flower bud ; i. e., formation of the terminal flower bud is easily influenced by the time of the high-temperature interruption, in other words, flower formation is greatly inhibited at the second half of 16-hour dark period than at the first half.

6. The first node of flower bud rises with increasing duration and degree of nyctotemperature ; that is, in 8- or 6-hour duration of the high-temperature interruption the first node of flower bud is influenced by the time of the high-temperature interruption ; the first node of flower bud rises as the last period of 16-hour dark period approaches to its close.

7. Number of plants with flower bud varies greatly, in 8- or 6-hour duration of the high temperature, by the time of the high-temperature interruption of 16-hour dark period ; while, in 4- or 2-hour duration of the high temperature not all the plants are influenced by the time of the high-temperature interruption ; i. e., all the plants have the flower bud.

8. The longer duration and the higher interruption temperature the more influenced is vegetative growth (dry weight per plant) of plant, while not all the plants are influenced by the time of the high-temperature interruption.

DETERMINATION OF PHOTOTHERMIC QUANTUM FOR THE VEGETATIVE PERIOD IN WHEAT

by J. J. Chinoy
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1. In a complex experiment involving 98 treatment combinations, unvernalized and vernalized seedlings (germinated at 27-20°C and 3-5°C respectively) of seven varieties of wheat (N. P. 165, N. P. 52, Punjab C. 591, Punjab 8-A, Cadia, Union, and Carlisle all belonging to *Triticum vulgare*) were transplanted under seven photoperiodic treatments (three short-7 hour day treatments of 45, 30, and 15 short days respectively, a normal day treatment, and three long-24 hour day treatments of 15, 30, and 45 long days respectively).

2. Lengths of the vegetative periods (number of days from the date of transplantation to the date of anthesis) were determined for 2450 plants (25 plants for each treatment combination) ; and the total number of light hours (photo quantum-P) as well as the sum of degrees of mean daily temperatures of the vegetative periods (thermic quantum-T) were calculated.
3. The relation between the photo- and the thermic quantum of a variety being given by a rectangular hyperbola the regression of the logarithm of the photo quantum on the logarithm of the thermic quantum of a variety was determined for unvernalized and vernalized plants for each variety individually. Positions of trend lines were determined by using the regression equations, and these were found to fit closely the experimental values.
4. Experimental values of photothermic quanta for all varieties were obtained by taking the products of the photo- and the thermic quanta for a variety under different treatments. These values agreed closely with each other for any given variety. The mean photothermic quantum of a variety also agreed closely with the mean of the products of the statistically computed values of photo quanta using the regression equations and the thermic quanta of a variety.
5. The vernalization quantum of a variety is the difference between the photothermic quanta of unvernalized and vernalized plants of a variety.
6. The photothermic quantum as well as the vernalization quantum of wheat varieties increase progressively with lengths of their vegetative periods.
7. The relation between the photothermic quantum (E) of a variety, the length of its vegetative period (F), the mean temperature (t) during the vegetative period, and the mean photoperiod (p) is expressed by the following equation :

$$E = PT = ptF^2$$

The vegetative period can, therefore, be calculated from the following equation :

$$F = \sqrt{\frac{E}{pt}}$$

If the vernalization quantum of a variety is large it should be taken into account in calculating the vegetative period of a variety.

8. Results of other workers have been discussed in the light of the concept of photothermic and vernalization quanta elaborated in this paper.
9. It is suggested that photothermic quantum can serve as a useful and a workable index of the energy requirement of a « long-day » plant.

ANALYSIS OF FACTORS DETERMINING THE YIELD OF WHEAT AS INFLUENCED BY PHOTOPERIODIC TREATMENTS

by K. K. Nanda
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1. Photoinductive and post-photoinductive treatments were given to three varieties of wheat (*Triticum vulgare :* var. N. P. 165, N. P. 52 and Punjab C. 591) for varying periods, and their effects were recorded on the grain and straw yields as well as the various characters determining grain yield, namely number of ears per plant, length of ear, number of spikelets and grains per ear, and thousand grain weight.

2. The number of ears per plant, length of ear, number of spikelets and grains per ear and thousand grain weight are all reduced under both short days (S.D.) and long days (L. D.).

The photoinductive treatments also affect the yield characters, though the effect is not so pronounced.

3. Grain and straw yields are also markedly reduced under both S.D. and L.D treatments. The reasons for the lowering of yields under the two light treatments are, however quite different. In S.D. treatment the reduction is directly due to the low rate of assimilation. In the case of L.D. plants, on the other hand, the effect appears to be due to the influence of light through regulatory mechanism of its developmental process.

4. The results of analysis of variance reveal that the single factor effects are practically all significant. Some of the interactions are also significant. Thus for instance photoinductive treatment N° 5 which results in a reduction in the yields of N. P. 165 and N. P. 52, increases the yield of Punjab C.591, which is a late flowering variety.

5. This increase in the grain yield of Punjab C. 591 under the photoinductive treatment N° 5 is considered to be partly due to the shifting of the ripening period of the variety in a range of temperature which is more favourable for the grain filling processes. The reduction in the yield of grain in S.D. plants was similarly ascribed to the shifting of the ripening period to a higher range of temperature.

6. Grain/straw ratio is also lower in S.D. and L.D. plants. This clearly indicates that the lowering in the grain yield in S.D. plants is not entirely due to a lower assimilatory activity but is also partly due to the adverse effect of the higher temperature of the ripening period in these plants.

7. Correlation coefficient between the mean maximum temperature of the ripening period and grain yield as well as thousand grain weight are, however, non-significant. This indicates that the effect of the maximum

temperature of the ripening period on grain yield is masked by some other factors operating in these experiments.

8. Correlation coefficients between the grain yield and various growth characters have been worked out. These correlations show that the masking of the effect of the temperature of the ripening period on grain yield in these experiments in brought about by growth characters, namely the number of tillers and plant high, which are reduced under L.D. and S.D. treatments. The reduction in these characters is reflected in the number of ears produced, length of the ear and also in the number of spikelets and the number of grains and ultimately in the grain yield.

9. Even after the effect of these yield characters are eliminated the yield per ear does not show significant correlation with mean maximum temperature of the ripening period. This is on account of the increased sterility of the pollen grain caused both by S.D. and L.D. treatments, which is reflected in considerable reduction in the fertility ratio.

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LIGHT, TEMPERATURE AND CHEMICAL FACTORS AFFECTING THE FLOWERING OF SUGARCANE

by George O. Burr

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In Hawaii the many varieties of sugarcane exhibit wide differences in flowering habits. Some do not flower at all, others produce a flower panicle on almost every mature stalk once a year. Most heavy flowering varieties are controlled by day length, some coming into flower in late summer, others not until late fall or winter. Other climate and soil factors have such a strong influence on flowering that within a short distance a variety may change from very heavy to zero flower production.

The following discussion applies only to variety 37-1933, a major commercial cane in Hawaii. Replicated tests, with and without light to inhibit flowering, show that in Hawaii's long crop system, heavy flowering may reduce yields from 10 to 20 %. These studies have therefore considerable commercial significance.

Floral initiation takes place in September, the buds reaching a size visible under the hand lens between the 20th and 30th of the month. The emerged flowers begin to appear in October and continue to come until February or March.

There is a fairly definite minimum age for flowering. April plantings will flower very heavily, May plantings will flower moderately and June plantings lightly or not at all. The day length and climatic conditions are so restrictive that cane not ready to initiate flower buds in September cannot flower until the following fall, when it will flower heavily at 15 months of age.

Sugarcane stools produce several stalks interconnected underground. There is free transfer of nutrients and organic materials between stalks, yet in a stool only one or a few of the stalks may flower, the others remaining in vegetative state.

Sugarcane behaves as a short day plant and flowering is inhibited by interrupting the night with artificial light. Incandescent tungsten light applied about midnight inhibits flower initiation in minimum doses of about 50 foot candle-minutes. This effect follows the reciprocity law over a considerable range of rates of application of light. The treatment must be continued for about 10 nights. The best time is between September 5 and 15. After this the light may be discontinued and flowering is permanently stopped for that year, even though the day length has changed only a few minutes.

Further shortening of the September day to less than 10 hours by rolling cars of plants into a dark house inhibits flowering.

Sugarcane is exceedingly sensitive to temperature. Changes of a few hundred feet in elevation may completely inhibit flowering. Plants in culture solution subjected to nights 5° C warmer or cooler than the natural temperature failed to flower.

High moisture conditions favor flower initiation. Partial withdrawal of irrigation water reduces the number of flowers without greatly reducing growth. Complete inhibition, however, requires extreme depletion of moisture over a long period which results in loss of most of the leaves and almost complete stoppage of growth. Vegetative growth is quickly resumed when water is again applied and flowering does not occur that year.

Soil quality has a striking effect on flowering. In tight heavy soils there may be no flowers, while in a nearby light alluvial soil flowers are plentiful. The plants in the two areas may be growing equally well with no striking chemical difference detected to date. The root systems are different in appearance and must be responsible for this great difference in flower production.

Heavy nitrogen fertilization will reduce the number of flowers but will not completely eliminate them.

Several chemical sprays reduce flowering when applied to the foliage in late August or early September. The most successful of these tried in Hawaii is Maleic Hydrazide. It can prevent all flowers without materially lessening growth of the stalk. Its effect is almost entirely in the growing point. All of the embryonic internodes immediately below continue to grow to approximately normal size in the weeks following application. The single application prevents flowers for that year.

A number of varieties of sugarcane do not flower at all in Hawaii. Attempts to induce flowering by changing day length or temperatures have thus far failed. No chemical sprays have been effective although coumarin and diiodobenzoic acid applied frequently over a period of weeks reduced the dominance of the terminal bud and induced growth of lateral buds. Similar effects have been produced with about 5.000 *r* of gamma rays from Co60.

INVESTIGATIONS ON THE PHYSIOLOGY OF GROWTH AND DEVELOPMENT OF *HIBISCUS SABDARIFFA*

by Sen Gupta and Sumitra Talukdar

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1. *Hibiscus sabdariffa* L.N.P. 5 was grown in pots at intervals of one month for eight months, commencing from 21st April in 1951, there being thus eight sowings.
2. The vegetative growth and development were greatly influenced by the time of sowing.
3. For widely different sowing times between April and August (120 days), the flowering took place within the end of September and the beginning of November (33 days) ; the vegetative period thus became progressively shorter with the lateness of sowing. The significance of the photoperiodic effect is discussed in this connection.
4. The growth in height, number of internodes and the circumference at the base of the stem became progressively shorter with the lateness of sowing, the sowings of September, October and November showing very poor growth.
5. The probable effects of the different environmental factors on the growth and development of the species have been discussed.
6. A favourable time for sowing *Hibiscus sabdariffa* L.N.P. 5 under the environmental conditions prevailing here seems to be between the months of April and May.
7. In another set of experiments, the seeds were vernalised at low and high temperatures for different periods, and in one experiment 1 ppm. Indole acetic acid was applied to the seeds in the low temperature treatment.
8. In every case, the presowing temperature treatments produced distinct latenesses in flowering from control, but there was no significant difference between the treatments themselves.

9. The addition of the hormone Indole acetic acid without effect on the flowering response.
10. In a third series of experiments the photoperiodic behaviour was investigated by (a) 8 hrs, 10 hrs, 12 hrs, 14 hrs, 16 hrs. treatments at the normal growing season ; (b) 10 hrs, 12 hrs, 14 hrs. treatments at three different seasons at intervals of three months for the effect of climatic factors of different seasons on the photoperiodic behaviour ; (c) treatment at 10 hrs, 12 hrs and 14 hrs. for periods of 14 and 28 days at the early stages for the induction effect ; (d) treatments at 10 hrs, 12 hrs and 14 hrs at the age of 30 days after manuring the plants with Ammonium Sulphate and Potassium hydrogen phosphate for the effect of the manures, and (e) 10 hrs, 12 hrs, 14 hrs treatments at the ages of 30 and 45 days, for the effect of age.
11. The plant shows distinct photoperiodic response.
    (a) *Hibiscus sabdariffa* L.N.P. 5 has been found to be a short day plant.
    (b) The climatic factors of different season influence the photoperiodic behaviour, but the effect is parallel to the general growth response of the plant.
    (c) There is a distinct induction effect.
    (d) Addition of Ammonium sulphate and Potassium hydrogen phosphate has no effect on the photoperiodic response.
    (e) The plants respond earlier after the commencement of treatment when they are 30 and 45 days' old.

EARLY FLOWERING OF DUTCH IRISES AS INFLUENCED BY TEMPERATURE AND LIGHT

by Annie M. Hartsema and Ida Luyten

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Early flowering of dutch irises (var. Imperator) can be obtained by planting the bulbs at a rather low temperature of 9°C after a previous heat treatment (1936, 1939, 1940). Shortness of natural light in some winters proved to be responsible for the failure of flowering in those years (1953). Contrary to many other plants, flower initiation occurs in the dark. Flowering begins (in a greenhouse at 15°C) towards the end of January, provided the amount of light during the last four weeks has been sufficient. In order to estimate the amount of light necessary for good flowering, investigations were made with artificial light only (luminescent tubes). Flowering proved to be in direct relation to the amount of light given in a 16 hour day. Later on we wanted to know whether there is a direct relation between flowering and day length. In 1952/1953 we found that by diminishing the duration of artificial illumination from 16 to 14, 12, 10 and 8 hours the flowering percentages remained between 86 and 100 %. In 1953/54 these experiments were repeated, but now by illuminating the plants during 10-8-7-6 and 5 hours daily. This time we see that flowering is in direct relation with time of illumination. Exact figures cannot be given on this date (Jan. 15 th). We suppose that even with these rather low light intensities (about 5000 lux) the assimilation rate is not proportional to the time of illumination. Dry weight estimations might conform this hypothesis. In our last publication we concluded that the effect of daylight in short winter days seemed to be more profitable for the early flowering of irises than the effect of artificial light in a 16 hour day. Therefore it is very interesting to see that about the same effect can be obtained by artificial light (of the same intensity) in a much shorter day.

References : Blaauw, Luyten and Hartsema : Early flowering of dutch irises. I. *Proc. Kon. Akad. v. Wet. (A'dam)*, **39**, 1936. II. *Ibid.*, **42**, 1939. III. *Ibid.*, **43**, 1940. IV. Hartsema and Luyten : *Ibid.*, **56**, 1953.

THE ROLE OF PHOTOPERIOD IN FLOWERING

by R. H. Roberts

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It is becoming difficult to appraise the role of photoperiod in flowering from a review of the literature. This trouble arises from such factors as differences in the interpretation of phenomena as well as of data. For example the practice has been followed in horticulture of giving plants a photoperiod classification as long- or short-day plants if they blossom earlier in one day length than another. If both lots come to blossom the plant should be classified as indeterminate. Also, photoperiod is sometimes considered to be responsible for *numbers* of blossoms produced. Actually, these are the result of vigor or rate of growth and not a primary matter of induction. In fact, the failure to distinguish an induced state from either a vegetative or a flowering condition has resulted in much confusion and misinterpretation. Two examples are particularly pertinent : (1) Interrupting the dark period when treating such plants as *Xanthium* does not really prevent blossom formation but only greatly delays it. That is, induction does take place but differentiation is greatly delayed. This fact markedly affects the philosophy on photoperiodism. (2) The conclusion that shoots arising from decapitated *Xanthium* plants which have been induced, are vegetative is not supported by anatomical evidence. Such shoots are from induced buds but form terminal inflorescences only after several weeks, in a manner similar to the flowering of plants having only one or two long dark periods. (A rose shoot arising from an axillary blossom bud may be some feet long and have many nodes but it was at no time a « vegetative » shoot though it had that external appearance when it was young.) Calling such growths non-flowering when they are young makes an understanding of the physiological mechanism of photoperiod effects very difficult.

A particularly unfortunate item affecting an understanding of photoperiodism is the common failure to distinguish between terminally flowering and systematically blossoming species. Plants of the former can be devernalized with a change in environment as vegetative growing points are present except near the tips and can give rise to non-flowering shoots. On the other hand, annual plants with all buds induced cannot, as a consequence, be returned to a vegetative type of growth. A full understanding of these situations is essential to the proper interpretation of what would otherwise be confusing data.

A frequently overlooked but very important factor in evaluating the effects of day length or, rather, night length upon flowering, is a proper appreciation of the importance of quantitative conditions in causing induction (or devernalization). This is illustrated by the usual need to defoliate a receptor plant when using the graft technique to induce flowering ; the foliage of the vegetative plant must be removed to permit a sufficient accumulation of induction stimulus from the foliage of the donor plant to be built up in the receptor. An original bit of evidence on the quantitative situation was obtained when vegetative plants of *Nicotiana tabacum*, var. T. I. 431, were grafted upon flowering plants of *N. glutinosa*. The very rapid and bulky growth of the scion caused blossom abortion on the stock and a return to a vegetative growth of the would-be donor instead of blossoming of the receptor.

The effect which photoperiod has upon other phases of plant development than blossom induction adds confusion to the problem of its role in sexual reproduction. The vigor or rate and type of growth as well as items such as numbers of flowers and set of fruits may be much affected by the length-of-day environment but are not a part of the induction or initiation of flowering.

When attempting to evaluate the role of photoperiod in inducing blossoming one is also faced with the situation that all but a few of the many plants which have been used in experimental work on photoperiodism can be induced with either temperature or age without regard to photoperiod. That is, day length is a truly controlling factor in flowering with only a small list of species and varieties. Could it be that the nature of flowering would be determined at an earlier date if less emphasis was given to photoperiod as a regulating environment and more to a direct consideration of the pertinent physiological and morphological aspects of floral induction ?

4. *LA THEORIE STADIALE DU DEVELOPPEMENT*

LA THEORIE STADIALE DU DEVELOPPEMENT

par
C. **Sironval** (*Liège*)
F. **Lanza** (*Rome*)
Cl. Ch. **Mathon** (*Paris*)
et M. **Stroun** (*Genève*)

Notre intérêt commun pour les questions relatives au développement des plantes et les conclusions analogues auxquelles divers travaux nous ont conduits, justifient la présente note qui exprime brièvement nos points de vue et confirme en ce qui la concerne la position adoptée par **Lyssenko** et son école.

A. Définition du développement.

Par développement d'une plante issue de semence, nous désignons le cycle des processus de formation des organes, le cycle des transformations qualitatives de la substance cellulaire, que la plante parcourt de la graine apte à germer à la graine apte à germer.

Il faut distinguer le développement d'une plante de sa croissance, caractérisée par l'augmentation de taille et de poids.

B. L'écologie des processus stadiaux, base de l'étude des phénomènes du développement.

1. — Le développement forme un tout régi par une loi fondamentale : celle de la stadialité. La plante accomplit nécessairement et irréversiblement divers stades pour se développer.

2. — L'accomplissement de chaque stade du développement exige un ensemble de conditions de milieu, correspondant aux propriétés de l'espèce. Selon les stades, l'une ou l'autre de ces conditions acquiert une importance relative plus grande. Dès lors, l'écologie du développement est avant tout une écologie des processus stadiaux.

3. — La succession des stades du développement peut être repérée par une série de critères d'ordre écologique, morphologique, biochimique ou autre. Ce serait une erreur de chercher à découvrir dans ces critères une illusoire « essence » du développement. Il s'agit, pour chacun d'eux, d'un indicateur, parmi d'autres, de transformations infiniment complexes, concentrées notamment aux points végétatifs des tiges.

4. — L'établissement des principaux critères marquant les stades du

développement s'appuie sur la connaissance des conditions écologiques correspondant à l'accomplissement de ces stades. L'écologie des processus stadiaux constitue ainsi la base de l'étude des phénomènes du développement en général.

5. — On peut définir les principaux critères stadiaux en utilisant les méthodes suivantes :

a) Critères écologiques :

L'application de la méthodologie de LYSSENKO (méthodes des semis échelonnés et de la modification échelonnée des conditions), et aussi de la technique de PIVNOVSKI, permet de reconnaître chacun des processus stadiaux élémentaires, chaque stade s'accomplissant plus ou moins rapidement selon le degré de réalisation des conditions qui lui correspondent (M., ST. : *Bull. Soc. bot. Fr.* 1953, L. : 1952). On peut également caractériser les différents stades successifs par leurs réactions à l'égard d'une seule condition précise du milieu (SIR. : Recherches organographiques et physiologiques sur le développement du fraisier à fruit rouge, 1951).

b) Critères morphologiques :

L'accomplissement de tel ou tel stade du développement correspond dans certains cas à tel ou tel aspect morphologique. On peut, par exemple, diagnostiquer, dans certains cas, l'achèvement des processus du photostade en se servant du critère de la forme des feuilles (SIR. : *Op. cit.*).

Cependant, il n'y a pas de rapport immédiat entre l'accomplissement des processus photostadiaux et la morphologie de la feuille (ou du point de croissance) ainsi que le montre, entre autre, l'analyse de la forme des feuilles à divers stades : l'accomplissement des processus photostadiaux crée seulement la base sur laquelle se constituera la nouvelle organisation du point de croissance (M., ST. : *Op. cit.* — observations sur *Triticum durum* et *Hordeum* div. sp.).

c) Critères biochimiques :

L'accomplissement de tel ou tel stade du développement est généralement repérable à une série de critères biochimiques. L'activité de la catalase et de la peroxydase est, par exemple, augmentée chez les froments ayant achevé le thermostade (L. : 1952 et Communication au Congrès). De même, l'achèvement du photostade chez *Fragaria vesca* est marqué dans les limbes par des rapports particuliers entre l'accumulation des pigments chlorophylliens et leur dégradation (SIR. : Communication au Congrès).

C. Les stades du développement.

Propriétés générales

a) Les différents stades se suivent dans un certain ordre. Un stade ne peut commencer avant que le précédent ne soit achevé.

Chez le Blé tendre « Vilmorin 27 », on ne peut obtenir d'épiaison, à partir d'un semis d'été sous lumière continue, que si la vernalisation a été achevée : l'accomplissement du thermostade précède et conditionne le photostade (M. : *Bull. Soc. bot. Fr.* 1952).

b) Les modifications stadiales sont irréversibles.
Quand une plante a achevé l'un des stades de son développement, elle a acquis, du même coup, un état qualitatif nouveau et il est impossible de la ramener à l'état qualitatif ancien.

1. En particulier, la vernalisation est irréversible. Le mot « dévernalisation » ne peut désigner des expériences au cours desquelles on empêche la vernalisation en intervenant avant son achèvement (Lyssenko : *Agrobiologie* — recueil —, Avakian et Iakstreb : *Agrobiologie,* 6, 1949, Dupéron : *C. R. Acad. Sc. Fr.* 1951, M. inédit).
2. De même, une fois le photostade achevé, la plante est dans un état nouveau nécessitant des conditions différentes.

c) Les exigences stadiales peuvent être relativement amples. Certaines plantes, par exemple, accomplissent rapidement leur thermostade à froid, mais peuvent aussi l'accomplir lentement à chaud dans les limites compatibles avec l'accomplissement de ce stade. Egalement aussi en ce qui concerne la durée du jour pour le photostade (Kornilov).

d) Les exigences stadiales sont spécifiques.
Les diverses variétés se distinguent entre elles, non seulement par leur forme, mais par leurs exigences à l'égard du milieu, à chaque stade de leur développement. Voir, par exemple, la série des fraisiers à gros fruits (Sironval) et les travaux de Lanza. La spécificité des exigences stadiales reflète la formation de l'espèce au cours des millénaires, en rapport avec le milieu.

L'ECOLOGIE DES PROCESSUS STADIAUX ET SON IMPORTANCE EN BIOLOGIE VEGETALE ET EN AGRONOMIE

par Lanza (*Rome*)
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Sironval (*Liège*)
et Stroun (*Genève*)

Les deux premiers stades.

a) Le thermostade

1. Pour l'accomplissement du thermostade, ou stade de vernalisation, le facteur déterminant est la température. Pour accomplir son ther-

mostade la plante a besoin, non pas d'un facteur isolé : température, humidité, lumière, obscurité, ou aliments minéraux, mais du complexe des facteurs nécessaires combinés dans des proportions convenables.

2. La transplantation embryonnaire chez les graminées permet de préciser que :

— le siège du phénomène de la vernalisation se situe dans l'embryon, comme l'indiquent LYSSENKO (*Agrobiologie* — recueil), GREGORY et PURVIS (1938) ;

— les produits élaborés par l'albumen lors du traitement au froid après trempage ne sont pas susceptibles de provoquer la mise à fleur de l'embryon transplanté. Ces produits activent le développement seulement lorsque l'embryon a déjà subi lui-même un traitement au froid (M. : *Soc. Linn. Lyon*, 1954 ; St. inédit). Par ailleurs, les embryons de Blés tendres (« Vilmorin 27 », « Vilmorin-Paris »), traités ou non par le froid, transplantés sur albumen de Blés durs (« Mahmoudi », « Xérès », « Médéa », « Battandier »), semés à la limite des conditions normales de l'achèvement de leur thermostade, donnent des plantes qui présentent, non pas une avance, mais un très net retard à l'épiaison ; le retard le plus grand s'obtient lorsque l'embryon transplanté n'a pas subi au préalable un traitement par le froid (M. : *id.* ; ST. *id.*). L'ensemble des faits infirme l'hypothèse de MELCHERS sur la « vernaline ». Il nous paraît exprimer que les « variations de la norme du type d'échange des matières nutritives provoquées par l'albumen étranger causent des variations dans le corps vivant » (LYSSENKO : *Op. cit.*).

3. Dans l'interprétation des diverses expériences sur la vernalisation, quelques tentatives schématiques faisant intervenir une succession de substances hypothétiques ont été faites, notamment par GREGORY et PURVIS et par MELCHERS. Ces schémas s'expliquent par le fait que leurs auteurs considèrent l'accomplissement des stades comme une stimulation, comme une méthode qui introduirait dans le processus normal du développement quelque chose d'hétérogène, de complémentaire, qu'il serait facile d'isoler, d'extraire chimiquement. De tels schémas éloignent de ce qui constitue la spécificité des rapports biologiques.

b) Le photostade

1. Pour l'accomplissement du photostade, la longueur du jour est le facteur déterminant.

2. La durée du photostade est délimitée par diverses méthodes (semis échelonnés et techniques inspirées de PIVNOVSKI). Chez les céréales, l'avancement du photostade n'est pas directement lié à des modifications du cône de croissance, mais tend à créer la base pour ces

modifications. Chez le fraisier, on observe que durant le photostade, le fonctionnement du méristème apical produit des feuilles de plus en plus compliquées, ce qui reflète la tendance à l'acquisition d'un état sur la base duquel se produira la mise à fleur.

3. Le méristème, en règle générale, ne perçoit pas lui-même directement la photopériode. Comme l'a montré Moshkov (*Bull. Appl. Bot. Gen. and Plant Breed.* 17, 25. 30, 1936), ce sont les feuilles qui sont sensibles à la durée des jours. Il ne fait pas de doute que des échanges de matières très complexes ont lieu entre le méristème et les feuilles — comme entre toutes les parties de la plante —, au cours du photostade du développement.

4. Cependant, l'essentiel dans le passage du photostade au suivant réside dans un changement qualitatif du type de fonctionnement du végétal considéré comme un tout. La théorie hormonale de Cajlahjan — et d'autres analogues —, ne correspond pas aux faits en ce qu'elle revient à identifier tout le développement de la plante à l'intervention d'une seule substance supposée florigène. Cette théorie est directement contredite par diverses expériences d'Avakian et de Prezent, ainsi que par les données récentes de Sironval sur les conditions de l'activité « florigène » de certains extraits (Communication au Congrès).

Croissance et développement.

1. Nous constatons, avec Lyssenko, que « la croissance est l'une des propriétés du développement des plantes » : il n'y a pas de développement sans une certaine croissance.

2. Les conditions du milieu n'agissent pas toujours de la même manière sur les deux processus ; les conditions nécessaires à la croissance, à un stade donné du développement, ne sont pas toujours identiques aux conditions nécessaires à l'accomplissement de ce stade. Ceci explique l'obtention dirigée de proliférations, fasciations et « néoténies » chez certaines graminées (M. : 1952, 1953, 1954 ; St. : 1953) et d'autres plantes.

L'importance de l'écologie des processus stadiaux en Agronomie et en Biologie végétale.

1. L'écologie des processus stadiaux est directement liée à la pratique agronomique. La culture rationnelle de la plante exige une connaissance approfondie des conditions de milieu nécessaires, à chaque étape du développement — Blés, Soja, Betterave — (Lanza, 1952 et inédit).

2. La description du végétal doit être complétée en tenant compte de ses relations avec le milieu à chaque stade de son développement. La définition de la forme, en soi, est insuffisante. La systématique

retirera de nouveaux points de vue de l'étude des propriétés écologiques. C'est ce que montre le cas des Orges étudié par **Mathon**, comme celui des variétés de fraisiers à gros fruits (**Sironval**).

3. En réalité, comme l'indique **Lyssenko**, « l'organisme et les conditions nécessaires à sa vie constituent un tout ». On ne peut reconnaître l'hérédité à la permanence de la forme dans n'importe quelles conditions ; le patrimoine héréditaire c'est « la propriété qu'a le corps vivant d'exiger des conditions déterminées pour vivre et se développer, et de réagir de manière définie à telle ou telle condition » (**Lyssenko** : *Op. cit.*), y compris la propriété de se transformer de telle ou telle manière sous l'influence de telle ou telle variation du milieu. — Voir en particulier les expériences de **Mathon** sur le maintien d'anomalies de l'inflorescence d'Orges obtenues par modification de l'achèvement du photostade (**Mathon** : *Soc. Bot. Fr.* 1954).
4. La théorie stadiale du développement, base nécessaire de la phénologie, facilite les mesures de protection parasitologiques.

THE DEVELOPMENTAL ANALYSIS OF LOCAL POPULATIONS IN THE CEREALS AND HERBAGE GRASSES

by J.-P. Cooper

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This paper presents an analysis of ecological differences between local populations, in terms of one important developmental system, the timing of inflorescence development on the shoot apex, and its corollary, the partition of energy between seed production and continued vegetative growth.

In the cereals and herbage grasses, leaves and spikelets are initiated on the shoot apex, within the leaf sheaths at the base of the shoot. Each potential node on the apex can give rise to a leaf with a bud in its axil. During vegetative growth, the shoot apex elongates slowly and carries only leaf primordia ; the axillary buds may later grow out as vegetative tillers. When head formation begins, apex elongation is accelerated, the leaf primordia are suppressed and their axillary buds develop into spikelets or panicle branches. Spikelet development is followed by internode elongation and eventual ear emergence.

This plan of inflorescence development is common to all Gramineae but its timing is under genetic control and varies with species and population.

Two conditions are necessary for spikelet initiation ; (i) the leaves must be exposed to suitable photoperiod, and (ii) the shoot apex must be competent to respond to the photoperiodic stimulus. Exposure to low temperature may be necessary for the attainment of such competence.

Greenhouse methods have been established for the rapid testing of response to photoperiod and low temperature. In the tests for competence, exposure to low temperature (vernalisation) is given to the seeds during germination, while in testing for response to photoperiod, differential photoperiods are applied as soon as the first leaf emerges. After varying lengths of initial treatment, all material is transferred to conditions optimum for floral development (in long-day plants, continuous light at 10-20° C.). Leaf number before heading on the main shoot is used as a quantitative measure of inflorescence development. It records the physiological age at which spikelet initiation occurs, and is unaffected by temperature fluctuations between 10° and 20° C.

The results of these tests indicate that response to photoperiod and to low temperature form important developmental mechanisms whereby adaptation to local climate and farming practice may be attained. Rate of attainment of competence determines the duration of the life cycle. In summer annuals, with a short growing season, such as the spring cereals, certain catch-crops (*Lolium westerwoldicum*), and many weeds (*Poa annua, Hordeum jubatum, Desmazeria sicula, Lolium temulentum*, and *L. remotum*) the shoot apex is competent at or soon after germination. When the growing season is longer, extending through a winter, biennials or winter annuals occur, such as the winter cereals, short-duration herbage crops (*Lolium rigidum, L. italicum*), and certain weeds (*Bromus arvensis, B. mollis*). In these plants competence is eventually attained without exposure to low temperature, but can be accelerated by cold treatment, either natural or artificial. In perennial herbage species, such as *Lolium perenne, Dactylis glomerata* and *Cynosurus cristatus*, few plants attain competence without low temperature, and the degree of perennity of local populations is related to their low temperature requirement.

Once competence of the shoot apex has been attained, photoperiod is usually the factor limiting spikelet initiation. Response to photoperiod determines season of flowering and is often related to latitude of origin. In such herbage species as *Lolium perenne* and *Dactylis glomerata*, the early-flowering hay strains have lower critical photoperiods than the late-flowering pasture strains, whereas in such ephemerals as *Poa annua* photoperiod is never limiting and flowering can occur at all seasons.

The above quantitative analysis of ecological behaviour in terms of the underlying developmental responses makes possible :

(i) the ecological classification of populations, and prediction of their behaviour under specified local climates and farming practice,

(ii) the rapid selection and progeny testing of parental material in plant breeding work,

(iii) an assessment of the potential genetic variation within populations, and the study of adaptive changes occurring under selection.

CONSIDERATIONS PHYSIOLOGIQUES, ECOLOGIQUES ET AGRONOMIQUES SUR LE THERMOSTADE ET LE PHOTOSTADE DES PLANTES HERBACEES

par F. Lanza

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L'activité de la catalase et de la peroxydase dans les graines de blé tendre alternatif (« Tevere ») pendant l'accomplissement du thermostade (vernalisation au frigo à 0°C) est plus grande que celle se manifestant dans les caryopses au germoir. L'activité de la carboanhydrase (= 900-1.000 mmc de CO_2 dégagé) et de la catalase durant le développement du blé « Tevere » vernalisé est plus importante que celle des témoins semés en automne et que celle des témoins semés au printemps (où toutefois le développement s'accomplit).

Chez les froments d'automne vrais (*Triticum vulgare* et *T. turgidum compositum*) semés au printemps sans vernalisation préalable, au contraire, le développement ne s'accomplit pas ou traîne et s'arrête ou est freiné au seuil du thermostade (carboanhydrase = 400 mmc de CO_2 dégagé). L'activité de la catalase, de la peroxydase et de la carboanhydrase éclaire la biochimie de la phase végétative d'une graminée, et rend compte — par rapport au traitement de vernalisation — du point de vue agronomique, du degré d' « automnalité » d'une variété.

Le comportement bisannuel ou annuel d'une plante normalement bisannuelle (*Beta vulgaris* et *Lactuca virosa*) dépend essentiellement de l'époque du semis, donc des conditions de l'accomplissement du thermostade et du photostade.

Par ailleurs, en déplaçant la date de semis de quelques variétés à long cycle de *Soja hispida*, de mai à août, on augmente la durée de la période levée-floraison et l'on raccourcit la durée de la période floraison-maturité ; tandis que chez les variétés à cycle bref, c'est le contraire qui se produit, ou, au moins, la durée de la première période reste la même. (Conséquences sur la durée du cycle et sur la production.) Les conditions de l'accomplissement successif du thermostade et du photostade agissent sur le végétal, déterminant un métabolisme particulier caractérisé par un profil biochimique spécifique. Chaque modification des conditions écologiques modifie le profil biochimique, modifie le développement et la croissance, laquelle est une propriété du développement.

LE PROCESSUS PHOTOSTADIAL CHEZ LES BLES DURS

par Maurice **Stroun**

Paris

Les Blés durs sont sensibles à la durée d'éclairement — comme facteur dominant — pendant une période du développement seulement. Cette dernière commence avant le début de la différenciation du primordium et se termine en tout cas bien avant l'achèvement de la formation de l'épi (Stroun, *Soc. Bot. Fr.*, 17, XI, 53).

L'accomplissement du processus photostadial n'est donc pas lié directement aux modifications morphologiques du primordium, mais crée la base pour ces transformations.

D'autre part, les primordia des plantes placées en jours courts, après avoir accompli le début du processus photostadial en jours naturels (15-16 h.), se développent plus rapidement que ceux restés en permanence en jours courts. Il y a donc un post-effet de l'action des jours longs qui ne peut s'expliquer que si « les stades du développement sont des changements qualitatifs dans le développement des plantes et non pas une formation d'organes et de caractères différents. C'est seulement sur la base de ces changements qualitatifs que se développent les organes et caractères des plantes » (Fédorov, *Agrobiologie*, 53, 3). Il va de soi alors que la réaction aux jours courts est différente de celle des plantes n'ayant pas subi ces modifications provoquées par les jours longs.

Les Blés durs — plantes de jours longs — peuvent se développer en jours courts (8 h.), mais très lentement. La différenciation du primordium se fait alors en présence d'une surface foliaire beaucoup plus grande. En effet, tant que le développement n'est que ralenti, la croissance se poursuit normalement. Par contre, si la durée du jour est assez courte pour arrêter tout développement, la plante périra, car il ne peut y avoir de croissance sans développement. « La croissance est une des propriétés du développement » (Lyssenko).

En accélérant ou en ralentissant les processus thermostadial et photostadial, nous modifions les rapports entre le développement et la croissance. Ceci nous permet d'obtenir à volonté dans le premier cas des plantes naines, dans le second cas un accroissement de l'épi sous la forme soit d'une ou de plusieurs nouvelles « bractées » à la base de l'épi, soit d'épillets secondaires, soit d'épis ramifiés (Stroun, *Soc. Lin. Lyon*, 54, I).

5. *RECHERCHES RECENTES SUR QUELQUES POINTS DE LA PHYSIOLOGIE DES FRUITS*

a) CROISSANCE

RAPPORT

AUXINES ET CROISSANCE DES FRUITS

par J. P. NITSCH

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En donnant du fruit une définition physiologique (46, 47), on simplifie d'un seul coup l'inextricable classification morphologique qui a prévalu jusqu'ici, on rend possible une vue synthétique des variations du fruit au cours de l'évolution (34) et, surtout, on concentre l'attention sur le fait principal : le fruit est un ensemble de tissus qui supportent les ovules et dont le développement est sous la dépendance de ces ovules. Cette définition met d'emblée en lumière l'action des ovules sur le fruit, action d'un organe sur un autre, exercée à distance par l'intermédiaire de substances chimiques définies et qui est donc, à proprement parler, une action hormonale.

I. — LA CROISSANCE DU FRUIT

Le développement d'un fruit est minutieusement réglé par une succession de stimuli d'après lesquels on peut distinguer trois grandes phases dans la période de croissance du fruit : (1) la croissance avant l'ouverture de la fleur ; (2) la pollinisation et la fécondation ; (3) la croissance après la fécondation des ovules.

1) *Avant l'ouverture de la fleur.* — Il y a bien peu de recherches récentes sur ce point, si ce n'est sur l'initiation même des tissus du futur fruit. Expérimentant sur *Bryophyllum*, RESENDE (55) conclut qu'un taux élevé d'auxine entraîne le développement de fleurs pourvues de pistils mais sans étamines. Chez certaines Cucurbitacées, l'initiation de l'ovaire peut être réglée à volonté par des traitements photo- et, surtout, thermopériodiques (48). D'autre part, l'application d'auxines synthétiques stimule grandement la formation de fleurs pourvues d'ovaires (fleurs femelles) chez le concombre (17, 18). Ces divers résultats font penser à l'existence d'un lien entre température et efficacité des auxines, interaction qui a été soulignée récemment (21, 22).

Une fois initiés, les tissus du jeune fruit se développent avec ceux de la fleur, principalement par multiplication de leurs cellules. La courbe de croissance dessine alors la première branche d'une « courbe en S ».

2) *Pollinisation.* — Lorsque la fleur s'épanouit, le jeune fruit devient incapable de continuer sa croissance tout seul. Il a besoin, alors, du stimulus de la pollinisation, puis de la fécondation des ovules. Autrement, la fleur fanée se détache et tombe, mettant ainsi une fin prématurée à la vie du fruit. Faut-il chercher dans la séparation du fruit d'avec sa source nourricière la véritable cause de sa mort ? Non, car même pourvue des éléments nutritifs qui permettent la croissance d'une fleur pollinisée, la fleur non pollinisée est incapable de pousser. La nouvelle technique de culture des fruits *in vitro* a permis de préciser ce point (45).

A ce stade, c'est le pollen qui joue le rôle décisif. On pensait, il y a quelques années, que le pollen stimulait directement la croissance par les auxines qu'il apporterait. Cependant, les extractions de pollen ne fournissent pas toujours beaucoup d'auxine (19), et les mécanismes enzymatiques proposés (39) à la place d'un apport massif d'auxines par le pollen ne semblent pas généraux (46). Faut-il incriminer les tests employés (courbure de coléoptiles d'avoine) ? Heureusement, nous disposons maintenant d'un nouveau test de parthénocarpie (26). Faut-il penser à une inactivation des auxines en cours d'extraction, ou bien à une grande spécificité des auxines du pollen ? Toujours est-il que l'auxine du pollen d'orchidée semble bien être l'acide indole-3-acétique (40).

S'il est encore difficile de mettre en lumière une action directe du pollen dans tous les cas, il ne reste plus guère de doute sur son action indirecte : le pollen fertilise les ovules et stimule leur développement. Ce sont eux qui vont assumer le rôle principal d'agents stimulants de la croissance du fruit.

3) *Après la fertilisation.* — L'effet exercé par les jeunes graines sur la croissance du fruit semble être un fait solidement établi maintenant. L'étude d'un matériel de choix, la fraise, a clairement montré que la forme ou le poids d'un fruit dépend de la position ou du nombre des ovules en développement. L'enlèvement de ces ovules à un moment quelconque de la phase de croissance active arrête net le grossissement du fruit. Les ovules contiennent une quantité importante d'auxines libres, et leur action sur le fruit peut être remplacée par des applications d'auxines synthétiques (44).

Des précisions supplémentaires ont récemment été apportées dans ce domaine. Le siège de la production d'auxines dans la jeune graine semble être l'albumen et non l'embryon (27). La compétition albumen-embryon expliquerait même que les variétés chez lesquelles l'embryon avorte avant d'avoir digéré l'albumen produisent plus d'auxines (29). La concentration d'auxines dans l'ovule varie au cours de son développement : un maximum très net a été enregistré huit jours après la fécondation chez le haricot (38), douze jours après chez la fraise (44) et environ vingt jours après chez le maïs (60, 72), tandis que Luckwill a relevé, chez la pomme, des maxima secondaires à côté du maximum principal (27, 29). Essayant de relier la production d'auxine aux événements physiologiques qui se produisent dans la graine, ce même auteur note que le premier maximum correspond au passage de l'endosperme du stade cénocytique au stade cellulaire et le second au développement d'un « albumen secondaire » (27). Tel est du moins le cas pour une auxine extraite par l'éther en milieu acide. Mais la situation semble être plus complexe puisque Luckwill vient de détecter jusqu'à quatre auxines différentes dans la pomme (31) !

L'action de l'ovule fécondé sur les tissus avoisinants est rapide. Chez les Fougères, le premier effet visible de la fécondation ne se trouve pas dans l'œuf lui-même mais dans les cellules du prothalle qui l'entourent. En effet, deux jours après la fécondation, on peut observer des divisions dans les cellules qui entourent l'œuf, divisions qui continuent pendant une quinzaine de jours. Au contraire, la première division dans le zygote n'apparaît que le cinquième jour (67). Murneek et ses élèves ont cherché à caractériser une hormone de syngamie à laquelle ils ont même déjà donné le nom de « syngamine », bien qu'ils n'aient réussi ni à isoler, ni à définir chimiquement cette substance. Au contraire, ils ont montré qu'un hydrolysat de caséine produisait les mêmes effets (33).

La parthénocarpie. — Si l'ovule joue un tel rôle dans le développement des fruits, comment alors expliquer la croissance des fruits sans graines ?

Tout d'abord, l'absence de graines dans le fruit *mûr* ne signifie pas grand-chose (56). En effet, les ovules ont pu être fécondés, les graines ont pu commencer à se développer, et avorter ensuite : il n'y a pas eu alors parthénocarpie. Car, de même que la parthénogenèse est la formation d'un embryon sans fécondation, de même la parthénocarpie est « le développement d'un fruit sans fécondation » (46). En évitant de confondre les expressions « fruit parthénocarpique » avec « fruit sans graines », on clarifie la question et on fait rentrer dans l'ordre de prétendues anomalies. En effet, si paradoxal que cela puisse paraître, il existe des fruits parthénocarpiques pourvus de « pseudo-graines » qui peuvent ressembler à s'y méprendre à des graines véritables. Des traitements aux auxines ont produit de telles pseudo-graines dans les fruits parthénocarpiques du houx (9), dans les agrumes (62, 63), le raisin (68, 69, 70), la figue (2), la pastèque (71) et même l'ananas (61). Des fruits provenant d'un développement parthénocarpique naturel peuvent aussi contenir des pseudo-graines (46). Ces pseudo-graines ne sont autres que les ovules du fruit qui n'ont pas été fécondés mais dont le développement a été stimulé par l'application d'auxines au moment de l'ouverture de la fleur. Si le traitement est effectué plus tard, lorsque les ovules ont dégénéré, on peut obtenir des fruits sans graines, des raisins par exemple (68). De plus, la nature de l'auxine employée a une influence sur la formation et la sclérification des pseudo-graines, en tous cas en ce qui concerne les pseudo-akènes de la figue (2).

Ainsi donc notre définition du fruit qui souligne le rôle de l'ovule n'est pas mise en échec par le cas des fruits sans graines. S'il existe des fruits sans graines, il n'existe pas de fruits sans ovules. Une très intéressante étude de la banane par SIMMONDS (58) confirme ces vues. SIMMONDS constate que les ovules des variétés parthénocarpiques de bananes sont de 10 à 25 pour cent plus grands que ceux des variétés à graines. De plus, ces ovules ont une vigueur plus grande, c'est-à-dire qu'en l'absence de pollinisation, ils augmentent en taille de 50 pour cent en une semaine, alors que ceux des variétés à graines n'augmentent que de 15 pour cent de leur taille initiale. La pollinisation des variétés parthénocarpiques de banane produit deux effets différents, suivant que des graines se développent ou non : (a) si des graines commencent à se développer, même si elles s'arrêtent ensuite, la taille du fruit augmente beaucoup ; (b) s'il n'y a aucune stimulation de la croissance des graines (cas des variétés hautement stériles), la pollinisation n'augmente pas la croissance du fruit. SIMMONDS en conclut que ce sont les jeunes graines et non le pollen qui stimulent la croissance du fruit.

II. — LES AUXINES DES FRUITS

Le pollen, les boutons des variétés naturellement parthénocarpiques (12) et surtout les ovules contiennent des quantités relativement élevées d'auxines. D'autre part, depuis les expériences fondamentales de GUSTAFSON (11), il a été maintes fois confirmé que les auxines synthétiques stimulent la croissance des fruits. Des recherches récentes ont été activement poursuivies dans ces deux directions.

1) *Les auxines naturelles des fruits.* — L'application des techniques de la chromatographie au domaine des auxines a grandement facilité la séparation et l'identification des auxines naturelles. Jusqu'à présent, comme dans tous les cas récents d'identification d'auxines extraites de plantes, ce sont des composés indoliques qui ont été caractérisés : acide indole-3-acétique (13), ester éthylique de cet acide (53) et acide indole-3-pyruvique (64) chez le maïs, acide indole-3-acétique dans le pollen d'orchidée (40). Les fruits immatures d'asperge semblent aussi contenir de l'acide indole-acétique (28), mais les jeunes graines de raisin et l'albumen des pépins de pomme contiennent une autre auxine (28) qui serait l'ester éthylique de l'acide indole-3-acétique (65). Une toute récente communication de LUCKWILL (31) annonce la séparation de trois auxines acides différentes plus une quatrième auxine neutre à partir de pépins de pommes. C'est dire que le problème est complexe, d'autant plus qu'à côté des auxines on trouve aussi des substances inhibitrices (28).

Quant aux précurseurs possibles de ces auxines naturelles, ils n'ont pas encore été démontrés sans ambiguïté. Une similitude de configuration chimique a longtemps fait soupçonner le tryptophane comme source des auxines indoliques naturelles. De fait, le taux de tryptophane augmente dans les fruits après la pollinisation (46), ce qui est en accord avec des études plus générales sur les variations du tryptophane au cours de la végétation qui notent une augmentation de tryptophane au moment de la floraison (1, 32) et une accumulation de tryptophane dans les cotylédons (14).

Enfin, il ne faut pas oublier qu'en plus des auxines et antiauxines existent encore dans les plantes des substances pouvant agir comme synergistes des auxines, par exemple les acides caféique et férulique présents dans la tomate (54).

2) *Les auxines synthétiques actives sur les fruits.* — Des travaux approfondis, portant sur un grand nombre de composés stimulant la parthénocarpie, ont été présentés par Wain et ses collaborateurs (8, 50, 59). D'une manière générale, les substitutions et isoméries qui diminuent l'activité d'un composé sur les tests d'auxines usuels diminuent également l'activité parthénocarpique. Il faut cependant noter une remarquable exception, celle de l'acide indole-3-acétique lui-même qui n'est que faiblement parthénocarpique. Peut-être est-il rapidement inactivé dans les tissus ? En tous cas, parmi les composés indoliques actifs, ce sont les esters éthyliques et méthyliques de l'acide indole-acétique qui viennent nettement en tête, suivis par les dérivés monochlorés de cet acide en positions 4, 5, 6 et 7 (57).

Parmi les fruits restés jusqu'ici les plus rebelles à la parthénocarpie induite par les auxines synthétiques, il faut citer les pommes, les poires et les fruits à noyaux. L'acide α-phénoxypropionique a donné des résultats encourageants pour la pomme (49). Sur la poire, les acides 2, 4, 5-trichlorophénoxypropionique (7, 10) et 2-naphtoxypropionique (49) semblent efficaces. Le cas des fruits à noyaux est plus ardu. Souvent, l'application d'auxines à la floraison entraîne la chute des jeunes fruits parce que ces produits tuent les jeunes graines. Mais plus tard, lorsque les graines sont plus résistantes, on peut obtenir une augmentation de taille et une avance de la maturité du fruit. Tel est le cas de l'abricot (3, 5), à rapprocher de celui de la fraise, chez laquelle un traitement à l'époque où l'auxine naturelle des akènes décroît produit une augmentation de taille supplémentaire de 30 pour cent (73).

3) *Comment les auxines affectent la croissance des fruits.* — En gros, les auxines agissent de deux manières sur la croissance des fruits : elles maintiennent le fruit sur l'arbre et elles stimulent directement le métabolisme du fruit.

Les auxines inhibent l'abscission des fruits à tous les stades du développement, de même qu'elles inhibent la chute des feuilles et d'autres organes. Cette propriété a été mise à profit pour retarder la chute des fruits aux approches de la cueillette. Mais au moment de la nouaison, ces mêmes auxines provoquent la chute prématurée des jeunes fruits. Ce résultat n'est paradoxal qu'en apparence. En réalité, l'acide naphtalène-acétique employé inhibe bien l'abscission des jeunes fruits. Mais, en même temps, il tue les graines, et c'est la destruction de l'albumen de la graine qui est responsable de la chute ainsi provoquée (42, 43, 30). Car, ainsi que l'a montré LUCKWILL (29), il semble bien que l'une des fonctions de l'auxine naturelle de l'albumen soit d'inhiber l'abscission du fruit. Il est remarquable que le phénomène de la « chute de juin » des jeunes fruits se produise avec plus d'intensité sur les arbres trop vigoureux et en périodes humides où la végétation est stimulée. Il semble qu'il existe une sorte de concurrence entre les points végétatifs et les fruits. Un tel point de vue expliquerait peut-être que l'ébourgeonnage de printemps stimule le développement parthénocarpique des figues (6). En tous cas, une concurrence entre fruits d'âges différents a été décrite autrefois sur le Cleome (41) et, plus récemment, sur l'okra (52). Un tout récent travail de MARRÈ (35) montre que c'est bien par l'entremise d'auxines que s'établit la concurrence entre fruits de divers âges, car l'ablation des jeunes graines la supprime et l'application d'auxine la rétablit.

De plus, les auxines stimulent directement le métabolisme du jeune fruit. La respiration augmente (16) ainsi que l'activité des deshydrogénases (35). De fait, TEUBNER et MURNEEK (66) ont décrit une stimulation directe des deshydrogénases malique et fumarique par les auxines dans les ovaires de tomates. Les auxines semblent aussi stimuler le mouvement des sucres vers les jeunes fruits où l'on observe une rapide synthèse d'amidon (36, 37). Enfin, elles stimulent nettement l'entrée d'eau dans ces tissus (15).

Sans vouloir entrer dans le détail des autres facteurs qui agissent avec les auxines pour faire croître les fruits, tels que les différents métabolites et co-enzymes (20, 23, 24, 25), mentionnons simplement une étude d'OSBORNE et WENT (51) montrant que l'efficacité des auxines varie avec les conditions climatiques, les températures basses et une grande luminosité favorisant la parthénocarpie tant naturelle que provoquée.

CONCLUSION

Peut-être n'est-il pas superflu, en terminant cette rapide et incomplète revue des travaux récents sur les auxines dans les fruits, de constater l'ampleur des développements économiques qui en sont résultés pour l'horticulture. Si l'emploi des auxines pour la mise à fruits est resté limité aux tomates de primeurs, par contre les pulvérisations d'auxines pour l'éclaircissage des fruits, la lutte contre la chute prématurée, l'avancement de la maturation, la coloration des fruits et, tout récemment, la sauvegarde des fruits pendant de légères gelées (4) sont des secteurs qui se développent et mettent en jeu des sommes considérables. Un tel succès atteste l'intérêt et la réussite des recherches dans ce domaine.

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RAPPORT

THE EMBRYO AND ENDOSPERM IN RELATION TO FRUIT DEVELOPMENT WITH SPECIAL REFERENCE TO *MALUS SYLVESTRIS*

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Our knowledge of the morphological and histological aspects of the embryo and endosperm of plants is fairly complete and quite detailed (17). The physiology of these important organs, connected with the development of most seeds and fruits however, is being explored only recently (37). The present interest in this field perhaps is due to our widespread and ramifying activities in plant genetics and to the recent theoretical interest and practical use, in connection with certain phases of fruit production, of the so-called growth regulating substances.

Most fruits require the presence of an embryo and endosperm for their setting and growth. Without pollination, fertilization and formation of the zygote(s), the ovule, ovary and associated tissues will not develop further and the flower usually abscisses. It is the object to discuss briefly here certain features of the relationship of the embryo and endosperm in fruit development with special consideration of the cultivated apple, *Malus sylvestris*. Not a few of the investigations that will be referred to have been conducted in the laboratories and orchards of the University of Missouri, U.S.A. Some of our results are still awaiting publication at the time of preparing this paper (23, 44).

Seed, Fruit and Food Relationships in the Apple

The polyembryonic apple fruit usually does not « set » unless fertilization has occurred in a considerable number of the 10 ovules. Under self-pollination, where self-fertility exists, the average number of seeds per fruit were found to be 3-5 or less, while with good cross-pollination it was 5-8 and up to 10. When the crop is fairly heavy, fruit with less than 3 seeds usually abscisses. There is a relationship not only between seed number and fruit size but also between leaf area and fruit volume. When the fruit set is relatively large then foliage becomes a limiting factor ; when it is light, seed number seems to determine size of the fruit.

Apples on shaded branches or trees, with the same fruit-leaf ratio, however, are larger in presence of a relatively high number of seeds containing vigorous embryos, as commonly happens from satisfactory cross-pollination. With good photosynthetic efficiency of the foliage, per contra, comparatively larger fruit will be produced in presence of the same or even fewer seeds (27, 29). Without the interference of other specific limiting factors, size of the apple, and probably other seed containing fruit, seems to be determined to a large extent by two major factors, the number of embryos and the available food supply. Let us now examine the function of the embryo and endosperm in fruit development.

Embryo and Endosperm Development

After fertilization the single-celled zygote undergoes, a « rest period », the length of which varies with the species, variety and environmental conditions (17). The endosperm usually develops to a multinuclear state before the fertilized egg divides for the first time.

According to some earlier and our histological observations, the zygote of the cultivated apple divides for the first time ca. 9 days after pollination. Subsequent development of the embryo is slow. On the 16th day it will have reached the 8-celled stage. Many abscissed fruit, about 2 weeks after fertilization, contained embryos in the 2 to 4-celled stage. Approximately one month after pollination the embryos were ca. 80-celled, the exact number being difficult to ascertain. In another month they were about 5 mm. long and filled the embryo sac. The now cellular endosperm had reached its maximal development and was soon absorbed by the embryo.

Later investigations have added the following major information on the subject (47, 41, 14, 44). At fertilization the endosperm consists of 5-6 nuclei. Hence formation of the primary endosperm nucleus, and its subsequent division, precedes syngamy. During the period of rapid embryo growth the now cellular endosperm is digested and replaced by a secondary peripheral endospermal tissue, which in turn is destroyed by the embryo (14). The uneven rate of development of the several embryos and endosperm in the same apple fruit suggests a variability in genetic and physiological states in the fertilized ovules (32). Some ovules, containing embryos, show such weak growth that they may even lag behind those containing no embryos (47).

Periodicity in Fruit Development

Periodicity in embryo, seed and fruit development of the apple has been observed in several investigations (41, 46, 14, 1, 15), which exhibits considerable similarity to that of stone (drupe) fruits of *Prunus*, since fruit of the genus *Malus* consists of 5 drupe like structures imbedded in the fleshy torus (45). Disconsidering growth of the unpollinated pistil, in which of course fertilization does not occur, there seem to be 3 stages in the development of the apple, the duration of each depending on the variety (28, 14).

During the first stage, lasting 3-5 weeks, the maternal tissue comprising the fruit develops rapidly, largely by cell division, but growth of the embryos is extremely slow. While the zygotes remain in a state of arrested development, forming small proembryos, growth of the endosperm is considerable, but it remains largely in the nuclear state.

The second stage may be recognized by a change of the endosperm from the free nuclear to the cellular condition and a simultaneous rapid growth of the embryo to full size. As a result the primary endosperm is digested by the embryo and, if a secondary endosperm is formed, it too is used up in the same manner (39, 38, 15). The duration of this stage may be relatively brief for early maturing varieties but quite prolonged for late maturing ones. The fruit tissue expands relatively slowly during this period and cell division seems to have ceased (1).

The third and longest stage is characterized by a rapid enlargement of the fruit itself to full size, which is almost entirely by cell expansion. There is no further growth of the endosperm and embryo but the seed, and its coat, undergo maturation and it enters the dormant state.

Studies in our laboratory point to the fact that similar changes occur in the tomato fruit (*Lycopersicon esculentum*). The comparatively brief period of protoplasmic growth of this fruit likewise ends when the embryo starts to develop and subsequent expansion is by cell enlargement (23). But in the pepper (*Capsicum frutescens*) the situation is somewhat different. Following fertilization the zygote remains in a state of rest for 1-2 days only. The endosperm becomes cellular before or at time of first division of the zygote. With the rapid development of the embryo, from the 5th to the 40th day after fertilization, there is a rapid expansion and digestion of the endosperm at the expense of nucellar tissue (6, 23).

Embryo Abortion in Relation to Apple Abscission

This subject has been studied mainly from observations of the absence or presence and amount of development of embryos in naturally abscissed young fruit. The ovaries start growing, by cell division, already in the flower bud. This enlargement ceases at anthesis (36). Without the stimulus of pollination and fertilization, the « fruit » abscisses. If these fruits (really flowers) are included and considered as the first extensive drop, then there are altogether four waves of abscission of immature apples, the first two usually overlapping (28). Fruit of the second drop show slight ovular growth, some endosperm but little (usually 2-4 cells) or no embryo development. Young apples persisting longer, but falling during the third wave of drops, show more endosperm and some (*ca.* 16 cells) embryo growth (8). In fruit, that absciss during the last wave of dropping endosperm development is quite complete, and embryos of various sizes are frequently present. However their total number per fruit is smaller and they are retarded in development in comparison to embryos in persisting fruit of the same age (44). For somewhat greater details of this subject we must turn to a related fruit of the genus *Prunus.*

Cytological studies of aborting fruit of the peach, var. Elberta, indicate that the cause of abortion most likely was a curtailed development and degeneration of the endosperm. As was emphasized before, nuclear division usually precedes that of the zygote in most fruits. In the retarded and eventually abscissing peaches lack of cell division and growth of the zygote was found to be due to non-multiplication of the endosperm nuclei (9).

Since embryos that are retarded, and would normally abort, can be raised artificially and often made to develop into seedlings, it would seem to indicate that an adequate supply of food or a necessary catalyst(s) seems to be lacking. This, too, points to endosperm as the cognate tissue responsible for the welfare of the embryo (45). While we do not know at present the cause or causes of the frequent endosperm inefficiency, it may be due probably to genetic difficulties associated with the 3n set of chromosomes.

The chromosome complex of the two participating gametes in sexual reproduction very likely play a crucial role in the fate of the endosperm and embryo, and consequently of a seed containing fruit. It is known that under self-pollination, where self-fertility to some extent exists in certain varieties of apples, the embryo and endosperm develop relatively slowly and early abortion of the fruit is frequent (4).

The observed cases of so-called « somatoplastic sterility » where there is an overgrowth of nucellar and integumental tissue, associated with embryo abortion, likewise seems to point to a lag in normal development of the endosperm (7). Artificial culture in proper media of somatoplastically inhibited embryos has been quite successful. The literature on culture of excised embryos has been reviewed recently (24).

Hormones in Relation to Fruit Growth and Abscission

Our present knowledge of the function of hormones in fruit development has been the subject of several recent reviews and therefore need not be gone into great detail (36, 37, 30, 31, 44).

The early growth of the fruit, up to anthesis, may possibly be due to either (a) a supply, through the pedicel, of a native hormone, probably 3-indoleacetic acid, from the vegetative parts or (b) it may come from the unripe anthers and/or ovules, both of which are high in auxin content, especially after synapsis (13, 33, 48). The latter source seems to be more likely, for when either the anthers or the ovules are excised, or otherwise, destroyed while still in the flower bud, then growth of the ovary is adversely affected (18, 19, 20). Moreover when the ripe male and female reproductive organs are relatively low in auxin content, as is the case at anthesis, the fruit ceases to grow (36).

It is well known that the rapid growth renewal of the fruit is subsequent to pollination, but particularly after fertilization and inception of vigorous embryos. High metabolic gradients are established in the ovules and ovary and food is mobilized and moved into the fruit even at a disadvantage to the vegetative organs (26). The catalytic mechanism bringing about these events is due to a rapid initiation of auxin production in the ovules. While in some fruits the relatively minor amount of free auxin formation, consequent to pollen tube growth, will stimulate fruit development, in the apple fertilization, preferably from cross-pollination, in a number of ovules must take place to prevent premature fruit abscission. As a result, free auxin diffuses from the ovules stimulating cell expansion of the fruit and preventing abscission. The conducting tissues of the pedicel are increased, thus facilitating food and water uptake (28, 25). Stimulation from fertilization seems to extend beyond the reproductive tissues affecting metabolism and growth of the whole plant (33, 48).

The specific mechanism of this catalytic effect is gametic union at fertilization (syngamy) and for growth of the ovary, previous to anthesis, very likely chromosome union (synapsis) immediately preceding reduction division (meiosis), which by some cytologists at least is considered as the end stage of sexual union (33, 48).

It is now quite well established that the abscission layer of the pedicel is under control of a native hormone formed in the fruit (15, 25). The periods of active natural fruit drops in the apple have been correlated with a relatively low hormone production in the seeds.

The suggestion has been made that an auxin-ethylene balance may be the immediate determining factor in abscission (10). Aging tissues, whether naturally or artificially brought about, like ripening fruit, may increase ethylene production which stimulates maturing of the abscission layer of the pedicel, probably through enzymatic action on the pectin compounds.

Attention has been focused recently on endosperm as the main tissue producing a native hormone in the apple seed. The peak of its formation seems to be associated with the transition in endosperm development from a free nuclear to the cellular state (14, 15). Why there should be an apparent subsequent decrease in hormone content is not clear. It has been suggested that « the natural function of this endosperm hormone in the apple might be the control of fruit drop » (14, 15). If so, then this is only of secondary importance for the primary well known function of endosperm is nutrition of the embryo (2, 3). In embryo culture studies, on artificial media, it has been observed that during the early stages of embryo development hormone-like substances supplied by the endosperm are indispensible (11). And despite the presence of endosperm, in absence of an embryo a seed-requiring fruit usually abscisses.

As in corn, so in the apple endosperm, the active hormone has been identified as the ethyl ester of indoleacetic acid (40, 43). It is possible that there may be a correlation between 3-indoleacetic acid and the ethyl ester of indoleacetic acid production and concentration in the endosperm and relative growth of the embryo.

Induction of embryo abortion with growth-regulating substances

A popular synthetic plant growth regulator, a-naphthaleneacetic acid (NA) has been used for a considerable time as a spray in the U. S. and elsewhere to prevent the premature dropping of apples and other fruits during the preharvest period. Now the same chemical, even at the same concentration, is employed successfully for removal of an excessive fruit set in the spring an artificial increase in abscission. These contradictory effects were puzzling indeed for some time.

Recent investigations have shown that an NA spray in the spring has a quite similar positive influence in retarding the abscission of fruit. This is expressed chiefly by a delay in the natural abscission of the young fruit of the second wave of drops, thereby making the third wave of abscission unnaturally extensive. But, in addition, NA has the stronger negative effect in the spring by inhibiting the development of the embryos. Evidently the young embryos and associated endosperm have a controlling influence on the abscission layer of the pedicel of the young fruit. This dual action of NA has been fully demonstrated by means of spraying defruited pedicels and by counts and measurements of embryo numbers and sizes (34, 31, 44). These results have been verified and additional information has been added to the effect that the period when NA is effective for fruit removal is during the time when the endosperm is in the free nuclear stage. As a result of embryo inhibition, growth of the fruit is also retarded (16, 44).

There may possibly be a correlation between retarded endosperm during the transition period to the cellular stage, embryo abortion and curtailed fruit growth. This is but another illustration of the powerful influence of the embryo on accessory tissue (fruit) development, which has been beautifully demonstrated with the strawberry (35).

An NA or a similar treatment with an effective growth regulator seems to have a primary effect on the endosperm (7, 42). In *Tradescantia*, for example, the application of a drop of a weak solution (10 parts per million) of 2,4-dichlorophenoxyacetic acid to sepals at various periods after fertilization inhibited endosperm to such an extent that it consisted of but a few nuclei even in relatively mature ovules. The collapse of the ovule followed, the embryo apparently being the last tissue to be affected as a result of the treatment, as judged by the capacity of cells to stain (42).

Whether as a result of an NA spray and consequent premature « aging » of the fruit, before it abscisses, there is an increased production of ethylene is not certain (44). It has been demonstrated that ethylene stimulates the dropping of leaves and other plant organs. There may well be an auxin-ethylene balance in operation.

Some similarities in metabolism from fertilization and application of growth-regulating substances

As was pointed out before, the ovary (fruit) at anthesis is a more or less resting organ. Following pollination and fertilization, and the consequent native hormone production, high metabolic gradients are established in the ovule and ovary. The transition is rather sharp and therefore favorable for metabolism studies.

The same series of events seem to occur when a fruit is stimulated into parthenocarpy by means of application of a synthetic growth regulating chemical such as α-naphthaleneacetic acid, 4-chlorophenoxyacetic acid or the ethyl ester of indoleacetic acid.

Recent biochemical investigations, conducted in our laboratories, with naturally fertilized and artificially produced parthenocarpic fruits show a rapid accumulation of nitrogenous and carbohydrate compounds as soon as growth is initiated (12). All ovulary tissues, especially the developing embryo, into which most of the nitrogen eventually goes, are high in proteins. The quite popular practice of supplying nitrogen in the spring to apple and other deciduous fruit trees, to increase fruit set, is quite in line with this requirement.

Information is meager and scattered as to the nitrogen metabolism in the young ovule and its essential parts. Our studies with young ovaries of the tomato show that protein synthesis was proportional to the increase in fresh weight during the first two days after a natural or artificial stimulation concomitant with fruit setting. Subsequently there was a dominance of water uptake, in this relatively « watery » fruit, as indicated by an increase in fresh weight over protein and dry matter (23).

The chief features of carbohydrate metabolism in naturally produced fruit during the first 6 or 7 days after fertilization, despite an increase in respiration, was a rapid accumulation of starch, a simultaneous decrease in sucrose and a progressive rise in reducing sugars. Treatments with an effective growth regulator, whether incorporated in lanolin and applied to the cut style (tomato) or sprayed in a lanolin emulsion (corn), produced quite identical results in carbohydrate metabolism and in proportion to the concentration of the regulator applied.

The mechanism for the increase and alteration in carbohydrate fractions must be sought in changes of hexose phosphates, due eitherto natural or artificial catalysis by « hormones » (22, 23, 36). As a result of fertilization or application of a synthetic growth regulator, there was an increase in organic/inorganic phosphorus and a significant augmentation of hexose phosphates. This despite the fact that they were used up at a rapid rate for growth. It would seem to indicate that their production was exceedingly high. This most likely was the result of a « hormone » induced activation of respiratory enzymes and their relation to the phosphorus transferring mechanisms (22, 23).

In view of these facts one may visualize the following chain of events as regards to the action of an NA spray to increase abscission of young apple fruits. When reaching the ovule it may uncouple oxidative phosphorylation. The first action may be a drop in the hormone content of the endosperm and subsequently reduced fruit growth. The second effect may be a retarded embryo growth coupled with a subnormal metabolism. The consequences of this may possibly be an increase in ethylene production leading to fruit abscission.

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THE AUXINS OF THE APPLE SEED AND THEIR ROLE IN FRUIT DEVELOPMENT

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The remarkable effects of synthetic growth substances in the induction of parthenocarpy, the prevention of abscission and in the ripening of fruits, strongly suggest that the growth and development of the fruit is normally controlled by naturally occurring growth substances, or auxins. Furthermore, the strong correlation which exists in many plants between seed development and fruit development would indicate the seeds as the most likely seat of auxin formation in the normal fruit. For this reason, in our studies at Long Ashton on the physiology of the apple, we have devoted special attention to the auxins which occur in the seeds an their possible rôle in fruit development.

At least four different auxins occur in ether extracts of apple seeds. These may readily be separated by the technique of paper chromatography using a butanol/ water/ ammonia solvent, and their locations on the chromatogram detected by means of biological tests (1). Three of these auxins are acidic substances and one is neutral. None of them appears to be identical with indolyl-acetic acid and nothing is known at present concerning their chemical nature. In addition to these four auxins the extracts contain two acidic growth-inhibiting substances, also of unknown chemical constitution.

The occurrence of these four auxins has been studied in relation to seed development which, in the apple, proceeds in three well defined stages (2), viz.

Stage 1, lasting 30 to 40 days from the time of fertilization is characterised by a rapid increase in the size of the seed, due principally to growth of the nucellus. During this period the endosperm remains in the free-nuclear condition and the fertilized egg-cell divides only very slowly to form a small spherical pro-embryo.

Stage 2. The beginning of this stage is marked by a very sudden change from the free-nuclear to the cellular condition of the endosperm (cytokinesis). Once this has occurred the embryo starts to grow rapidly and the cotyledons, plumule and radicle are differentiated. The primary endosperm is digested and replaced by a secondary endosperm developed from a peripheral meristem.

Stage 3 is a period of maturation in which no further growth of the embryo or endosperm occurs. There is an accumulation of food materials in the embryo, and the testa hardens and turns brown.

The distribution of auxin in the seed at these three stages of development is shown in the following table :

		Acid auxins			Neutral auxin
Stage	**Condition of seed**	**1**	**2**	**3**	**4**
1	Endosperm free-nuclear	+	++	+	—
2	Cytokinesis in endosperm	+	+	++	+++
2	Embryo growing rapidly	+	+	+	++
3	Completion of embryo growth	+	++	+++	—
3	Seeds mature	+	++	+	—

Auxin 1 is found in the seed at all stages of development and does not appear to be correlated with any particular phase of seed or fruit growth. Its function is at present unknown.

Auxin 2 is of particular interest because it is the only one of the four seed auxins which has been found in any quantity in the fruit flesh. It appears in the fruit shortly after fertilization and thereafter can be found at all stages of development, being particularly abundant in the mature fruit at harvest. In Lord Lambourne apples infected with chat fruit virus, which arrests the growth of the fruits at any early stage and causes them to remain hard and green, this auxin was found to be absent from the fruit flesh. This observation suggests that auxin 2 may be associated with the growth and ripening of the fruit. There is no evidence at present as to the seat of formation of this fruit auxin. It could originate in the pith or cortex of the fruit, but it seems more likely that it is translocated either from the seeds or from adjacent leaves on the fruit spur.

Auxin 2 is also thought to play a part in controlling the dormancy of the apple embryo. The mature dormant embryo contains no free auxin, but if the seed is after-ripened by storing under moist conditions at 4°C for 50 to 60 days, there is a reappearance of auxin 2 in the embryo, which is now capable of rapid germination.

The acid auxin 3 shows a close association with endosperm development. During the free-nuclear stage it is present only in small quantity. It shows a marked increase at the time of cytokinesis and thereafter a decrease as the primary endosperm is absorbed. With the formation of the secondary endosperm auxin 3 again increases, reaching its peak at the time of completion of embryo growth. During stage 3, the endosperm degenerates and the amount of auxin 3 in the seed again falls to a low value. Detailed study of this auxin throughout the season, using the tomato parthenocarpy test, in which it is particularly active, has shown a close correlation between the peak periods of auxin 3 production and the time of cessation of the first and second waves of fruit drop (3). On the basis of these results it has been suggested that the natural function of this auxin is to control the abscission of the developing fruit.

The neutral auxin 4 is found in the seed for only a short period. It first appears at the time of cytokinesis in the endosperm and soon disappears again as the embryo enters its period of rapid development. These facts suggest that it may be the factor which stimulates this sudden and rapid growth of the embryo, though there is at present no direct experimental evidence of this.

This work is still in its early stages and many problems remain to be solved. It is clear, however, that in the growth of a fruit such as the apple a number of different auxins are involved. One of these may control fruit growth, another the abscission process, whilst others appear to be connected with growth processes within the seed itself.

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b) MÉTABOLISME

RAPPORT

THE CONTROL OF RESPIRATION AND SYNTHESIS IN APPLE FRUITS

by Judith A. PEARSON and R. N. ROBERTSON

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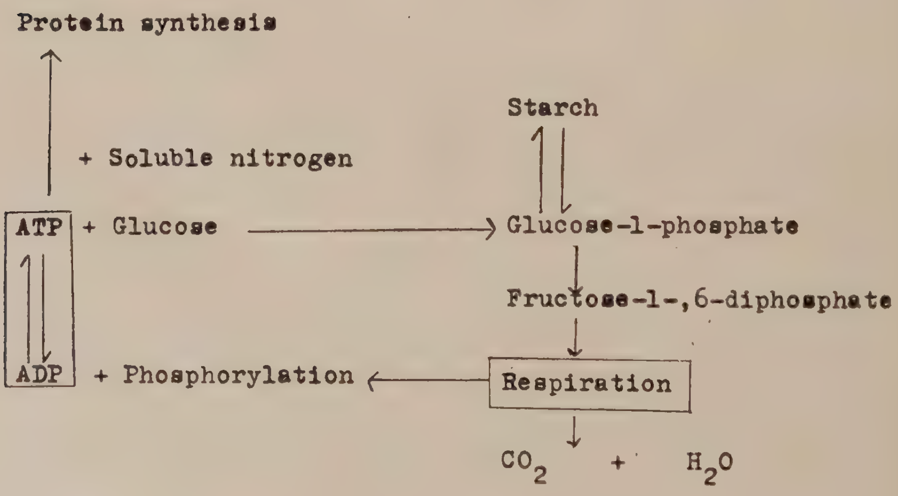
The subject of this symposium, the role of certain protoplasmic constituents in the climacteric phenomenon, raises the question of the mechanism of control of rates of synthesis and respiration in fruits. After some years work on apple fruits (BAIN and ROBERTSON (1951), ROBERTSON and TURNER (1951) and PEARSON and ROBERTSON (1953)), we were impressed with the need to account for the disappearance of starch during the time of rapid increase in respiration rate after the fruit had reached normal maturity. Following some unpublished lectures of MASKELL (1948), it had been suggested (ROBERTSON and TURNER 1951) that the relationship between these processes should be sought in the phosphate transfer system which is considered to play a controlling part in the respiratory and synthetic processes. The hypothesis for the mechanism of this control has been discussed to some extent by LARDY (1952) and by MILLERD and BONNER (1953). The hypothesis may be summarised here by reference to the following simplified scheme :


Protein synthesis
+ Soluble nitrogen
Starch
ATP
+ Glucose
Glucose-1-phosphate
Fructose-1-,6-diphosphate
ADP
+ Phosphorylation
Respiration
CO_2 + H_2O

It is now thought that probably about 70 % of the energy of respiration passes through the phosphate carrier system with the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) by the phosphates coming from the intermediates of respiration. Since the oxidative steps in normal respiration cannot proceed without the simultaneous phosphorylations (i. e. phosphorylations are compulsorily coupled with oxidations) the respiration rate is governed by the amount of ADP available to accept phosphate. This in turn is dependent upon the rate at which ATP is used in the various cell processes including synthesis of proteins for growth and for maintenance, and synthesis of starch and of other compounds. If ATP is used rapidly then ADP will be regenerated rapidly and respiration will proceed as fast as the oxidations allow. The regeneration of ADP depends on the phosphate requiring processes and the rate of any of these processes depends on the amount of ATP available and the competition between the ATP dependent processes. Thus the phosphate carrier system and the ratio ATP/ADP provides a mechanism linking respiration and synthetic processes and offers a hypothesis of how the rates of the processes are connected.

An analysis of apple tissue in different stages in the development of the fruit to determine the ATP and ADP directly would be very valuable in assessing this hypothesis. Unfortunately this has been difficult until recently and techniques have only just been developed. Meantime it has been possible to obtain some indirect evidence for this hypothesis. This evidence will be presented in this paper and has been discussed at greater length elsewhere (PEARSON and ROBERTSON, 1954).

First let us examine the changes in developing apple fruits of the experimental variety (an Austràlian variety — the Granny Smith).

Physiological and anatomical studies have been made on developing and mature Granny Smith apples over seven seasons from 1947-1953. The samples were all taken from the same source. Changes in the following factors have been followed : — Cell size, cell number, fruit size, respiration rate, protein and soluble nitrogen, total sugars, starch, organic acids, cut-tissue respiration rate, effects of 2,4-dinitrophenol (DNP) and adenosine triphosphate (ATP) on the cut-tissue rate and the activity of some enzymes found on isolated particles.

The life of the Granny Smith apple can be studied in four stages. At pollination the receptacle contains about 1,000,000 cells. During the next 3 - 4 weeks (28 days) these cells divide to form an average of about 40,000,000 cells. After this time there is no further cell division and the second stage is one of cell enlargment accompanied by changes in composition and respiration rate. This continues for about 5 months (180 - 190 days) in the Granny Smith variety. The third stage is marked by a sharp climacteric rise in respiration rate and a sharp increase in soluble nitrogen. This stage cannot be given any definite time as its duration varied from season to season. In the final stage the respiration rate continues to rise but not at such a rapid rate and further changes in cellular composition occur. If the apples are undisturbed they remain on the tree for 3 - 4 months (until 270 days from full blossom) after they are commercially ripe, at the climacteric rise, and remain very active physiologically.

Some of the changes which take place in the cells are represented diagrammatically in Fig. 1 and will be described briefly. In this figure all cell constituents are expressed on a per cell basis and are plotted against days from full blossom which is the time scale used throughout the work.

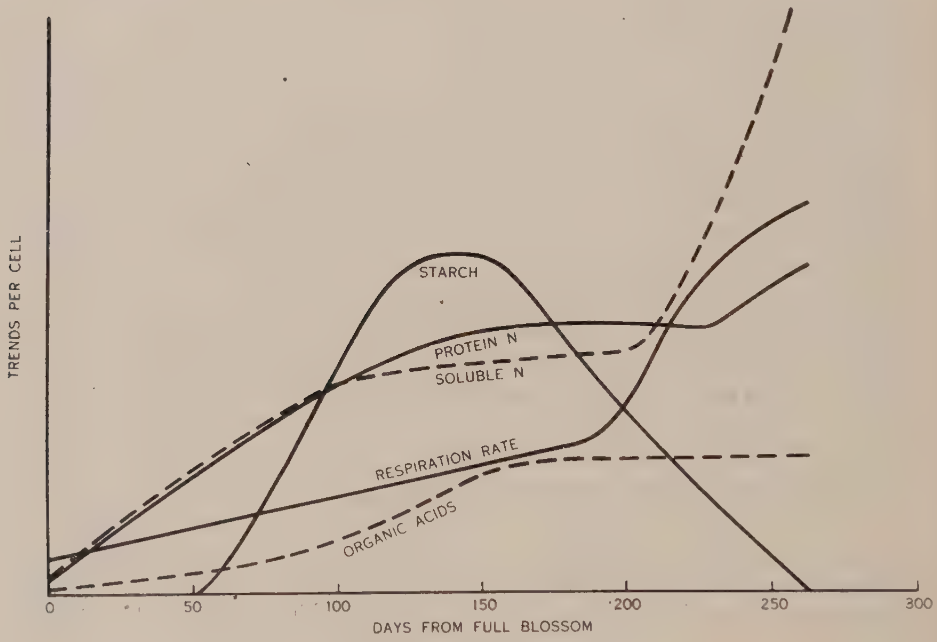


Fig. 1.

(a) Respiration rate per cell increases with cell enlargement in a rate proportional to the increase in protein nitrogen per cell. At 190 days, which is about the date of commercial picking, a sharp rise in respiration — the climacteric — begins. The respiration rate continues to rise for the rest of the season but at a slower rate after about three weeks. We have never had any suggestion of a falling off in respiration rate in the climacteric on the tree.

(b) Starch appears in the cells at about 50 days from blossom and increases rapidly to a peak at about 120 days. It changes very little until 165 days when it begins to decrease again very rapidly to nil at 230 days. Starch is breaking down in the cells for about 4 weeks before the climacteric rise begins. The sugar produced by the hydrolysis of starch is only a small fraction of the sugar coming into the fruit at this time. Total sugars increase from 24 % of the dry weight in the young fruit to 71 % at the time of commercial picking.

(c) Soluble nitrogen increases in the cells up to about 120 days. After this time there is a lag when the soluble N is maintained at a constant level. At 210 days there is a sharp rise in soluble nitrogen which continues to the end of the season.

(d) Protein nitrogen is present in equal amount to soluble nitrogen for about 120 days but continues to increase very little more and then decreases again after 160 days. However at 230 days new protein is again synthesised and there is an increase in the protein in the cells at the end of the season.

(e) Organic acids are interesting in that, as found by KIDD, WEST, GRIFFITHS and POTTER (1951) they are not affected by the climacteric rise. Total acids increase in the cells up to 150 days from blossom and then remain at much the same level till the end of the season.

After the changes in cell composition during the life of the Granny Smith had been fairly well established and the time of occurrence and nature of the climacteric rise was known, the investigations turned to a study of the controlling factor in respiration rate and to the control of some of the synthetic processes. As it was not possible to use direct methods on whole fruits, we have studied the respiration of cut tissue and the effect of added substances. In addition a crude mitochondrial preparation has been used to follow some of the enzyme changes.

The mitochondrial suspension was prepared by blending the tissue in sucrose and centrifuging a muslin squeezed homogenate at 1150 g for 20 minutes. This partial suspension was tested in a Warburg apparatus with substrates of sodium succinate, sodium citrate and sodium malate. The results are shown in Fig. 2. The activity is expressed as oxygen uptake per mg. nitrogen in the preparation and plotted against time. The activity with each substrate remained fairly steady up to 190 days. When the respiration of whole fruit increased in the climacteric rise there was a marked increase in activity of the particles which continued for as long as we took samples. The activity of the preparation without substrate decreased towards 150 days, which may be a reflection of the decrease in concentration or organic acids in the tissue, but the marked rise in activity at 190 days occurred even without substrate.

Increased activity per mg. of nitrogen in the particles, implies either that the oxygen uptake by the particles had been controlled by some factor such as the ATP/ADP ratio in the particles, which changed at the time of the climacteric or that the enzyme concentrations had increased relative to the other nitrogen constituents. The second possibility would seem to be less likely than the first since increase in the concentration of the oxidative enzymes would be expected to cause a sharp rise in the respiration rate of cut tissue in 2,4-dinitrophenol ; as will be seen later a rise did not occur at this stage. Control of oxidation rate by the ATP/ADP in the extracted particles is consistent with the general hypothesis of control in the intact tissue which will be explained later.

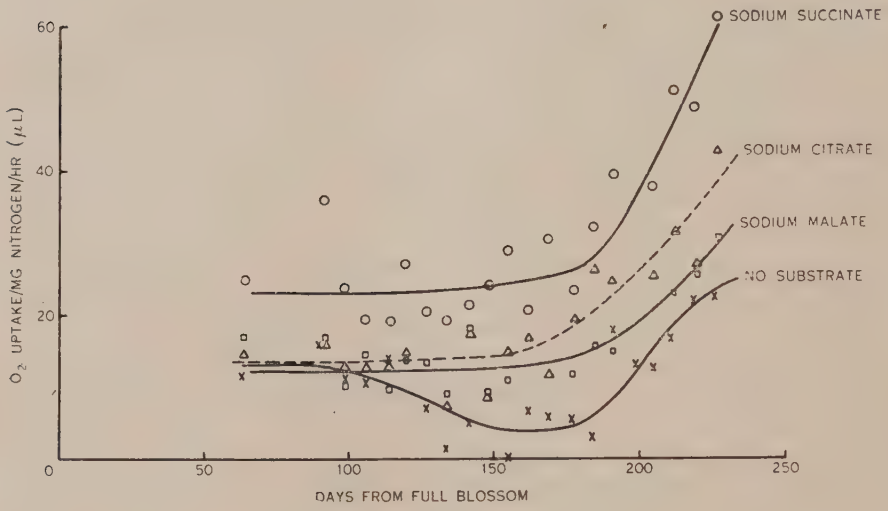


Fig. 2.

It was shown earlier that the total organic acid content of the fruits did not change at the time of the climacteric rise. The increased activity of the enzymes concerned with an acid cycle, during the climacteric rise, indicates either that the gross concentration does not relate very closely to the respiratory activity or that there is an increased rate of turnover in acid without any change in net content. The occurrence of enzymes in the particles, capable of oxidising three acids, succinic, malic and citric is further evidence for the participation of a tricarboxylic acid cycle in apple respiration. This is supported by recent work which has shown that the particles can also oxidise pyruvic, fumaric, and α-ketoglutaric acids as substrates. The increase in activity of the particles with the increase in respiration rate suggests that these enzymes can meet the extra demands placed on them by the climacteric rise.

Cut-tissue respiration rate was measured by placing discs of cortical tissue 1 mm. thick into an isotonic solution of glucose and phosphate in the Warburg apparatus. The average rate was measured as oxygen uptake per g. fresh weight per hour. To compare this with the respiration rate of whole fruit, the cut-tissue rate has been multiplied by the whole fruit weight. Since we know from earlier work that cell division is complete within 30 days from blossom, the change in respiration rate per fruit should follow the same course as the change in respiration per cell. Cut-tissue and whole-fruit rates are plotted against days from full blossom in Fig. 3. In the early samples the respiration per cell of cut tissue and of whole fruit are similar but by 100 days the cut-tissue respiration per cell is about double that per cell of the intact fruit. After about 190 days when the whole-fruit respiration showed a climacteric rise, the cut-tissue respiration rate also increased in both seasons.

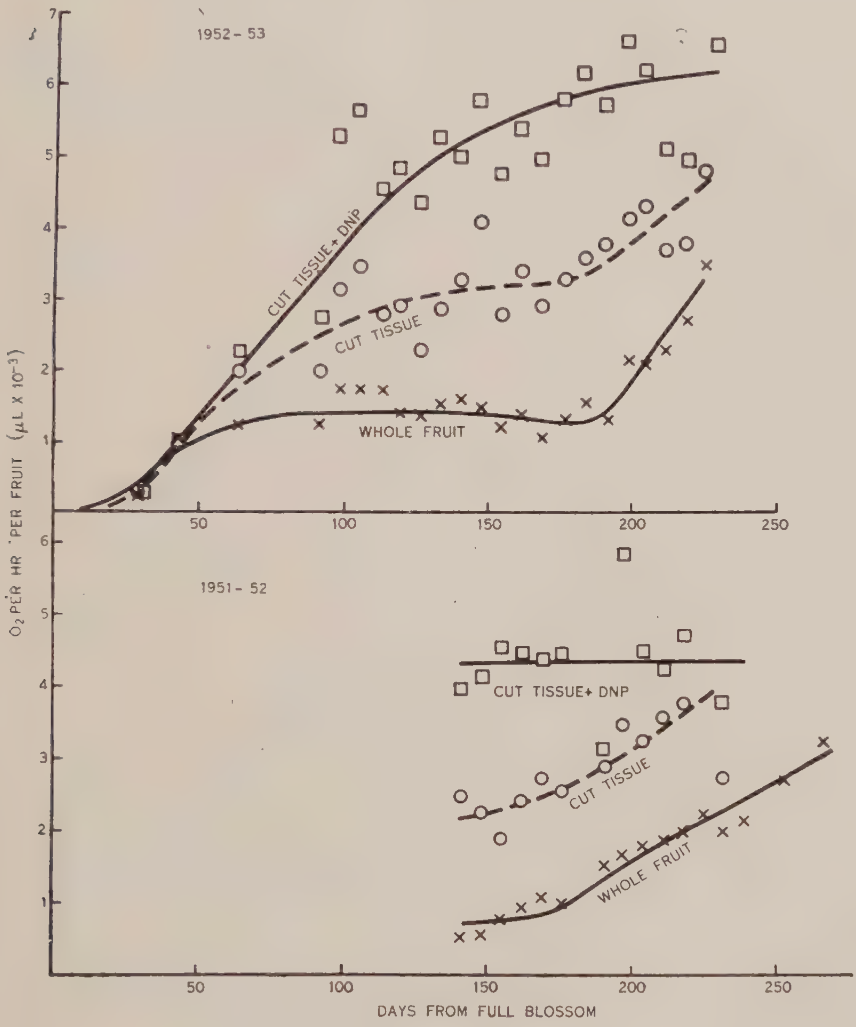


Fig. 3.

The substance 2,4-dinitrophenol is known to uncouple phophorylation while allowing oxidation to proceed. It was postulated that if respiration rate was controlled by the concentration of ADP and if the total adenosine polyphosphate was not changing, DNP would cause a stimulation of respiration rate where the ratio ATP/ADP was high in the pre-climacteric stage and would have very little effect at the climacteric rise where the ratio was low. The results with added DNP support this hypothesis.

2,4-dinitrophenol was added to the cut discs of tissue in a concentration of 0.5 mg/litre throughout the experiments. These results are shown in Fig. 4. In the early samples up to 60 days from blossom the effect of adding

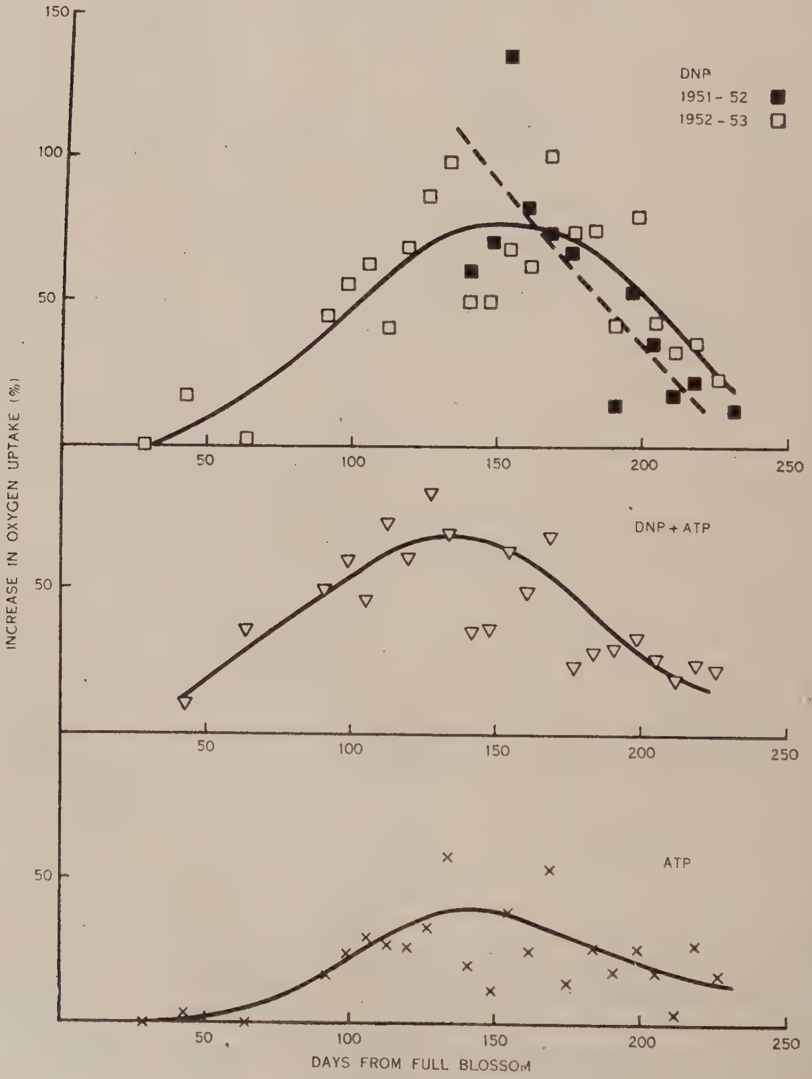


Fig. 4.

DNP was small. Up to this time ATP is probably required for active protein synthesis and synthesis of other compounds necessary for growth, the ratio ATP/ADP would be low and little effect of DNP would be expected. From 60 days onwards the stimulatory effect of DNP increased. Here the respiration rate was probably controlled by the decreasing ADP concentration as the rate of phosphorylation by respiration exceeded the rate of utilisation of the ATP thus formed. Further evidence for this interpretation comes from the increase in starch which is being rapidly synthesided in the cells, indicating that the ratio ATP/ADP is now high, the phosphorylations in excess of those required for growth and maintenance going to the formation of glucose-1-phosphate which is being condensed to starch. Both starch content (indicating high ATP concentration) and DNP stimulation (indicating low ADP concentration) reach their maxima at about 130 days. After 160 days no more starch is synthesised and it begins to breakdown. The protein level in the cells at this time is only just being maintained. (Fig. 1). McKee and Urbach (1953), working on the same apple samples, found that glutamine synthesis ceased at about 138 days from blossom and glutamine disappeared from the tissue, suggesting a shortage of ATP required for its synthesis. From about 150 days, the general decrease in synthesis indicates that ATP is now in short supply for these processes. The simultaneous increase in ADP would tend to increase the respiration rate and to lower the percent. increase in respiration due to DNP. At a critical stage a small change in the ratio ATP/ADP produces a large increase in respiration which is the climacteric rise, beginning here at about 190 days.

As the fruits went through the climacteric rise, the oxygen uptake per cell of cut tissue increased also, but the oxygen uptake per cell in the presence of DNP increased only slightly with increase in cell size, i. e. it showed no climacteric rise (Fig. 3). This suggests that the ATP/ADP ratio remains in control of the respiration at all stages but a change in ratio allows an increase in both whole-fruit and cut-tissue respiration after 190 days.

The hypothesis that the ADP concentration limits the rate of respiration in fruit from about 65 days to 190 days is further supported by results from added ATP. ATP was added to the cut tissue in a concentration of 3 mg/ml and the curve in Fig. 4 produced. The hypothesis that the ATP/ADP ratio controls the respiration rate is based on the assumption that the amount of adenosine polyphosphate which occurs naturally in the cells does not alter rapidly. However, if the absolute amount is increased by the absorption of ATP supplied in the external medium, the amount of ADP will be increased even though the ratio remains the same. A trend similar to DNP was obtained.

The simultaneous addition of ATP and DNP gave a curve similar to that obtained with DNP alone (Fig. 4). The fact that the stimulation due to DNP added with ATP was not greater than that due to DNP alone indicated that the uncoupling of respiration from phosphorylation by the DNP was complete, since addition of more phosphate carrier did not increase the oxygen uptake.

The rate of respiration has been increased in 4 ways : 1) by the climacteric rise, 2) by cutting, 3) by DNP, 4) by ATP. We have suggested that one of the most important factors controlling the respiration rate is the phosphate carrier system and respiration is increased in each of the above ways by producing some change in the concentration of ADP, thus altering the ratio. The events leading to the increase in rate by the climacteric rise have been discussed. The increase in rate on cutting the tissue may be due to a number of causes such as a shearing and squeezing effect on cells associated with the cut cells. Whatever the cause, any effect of cutting likely to lower the ATP/ADP ratio would raise the respiration rate. Such factors may be the increased demands of synthesis as the cells adapted themselves after cutting or the increase in activity of an ATP-ase liberated. However, as we have shown, respiration rate of the cut tissue is still under the control of the ATP/ADP ratio to some extent since stimulation by DNP is produced. Respiration is also increased by increasing the amount of ADP available.

Assuming that respiration rate and synthesis are controlled by the ratio we can look at the 4 stages of development again. In the cell division stage protein is being synthesised actively and there is no DNP or ATP effect. The ATP would probably all be going towards protein synthesis leaving very little for other syntheses and the ratio ATP/ADP would be low. During the early part of the cell enlargement stage protein is still being synthesised in the enlarging cells but less rapidly and some of the ATP is available for other syntheses. Starch begins to increase in the cells and as it increases to its maximum the DNP and ATP effects reach their maxima. The ratio here is high. As cell enlargement proceeds the protein synthesis does not keep pace after a while and it appears that synthesis and maintenance are now difficult. Soon after this time the starch begins to decrease indicating that the ratio is decreasing still further. Respiration rate has been increasing slowly as the ratio decreased, but at 190 days when the ratio has reached a low level, respiration rate increases sharply in the climacteric rise. However even though the respiration increases, starch continues to decrease and protein does not increase for a while. For some time ATP is inadequate but the final increase in protein indicates that some ATP is again available for synthesis.

Conclusion. — The evidence presented from the results of the experiments described, supports the hypothesis of the control of respiration rate and synthesis through the phosphate carrier system. The hypothesis opens the way for further investigation by development of methods to estimate the ATP/ADP ratio directly. The hypothesis is of value, both as a starting point for further work and as an attempt to present a unifying hypothesis.

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OXIDATIVE PHOSPHORYLATION BY CYTOPLASMIC PARTICLES OF FRUITS IN RELATION TO THE CLIMACTERIC

by Jacob B. **Biale**

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The successful preparation of metabolically active cytoplasmic particles, as outlined in this paper, has stimulated a new attack on the unique phenomenon in fruit physiology, the climacteric rise in respiration. The progressive decline in the oxygen uptake of the intact fruit until the preclimacteric minimum is reached suggested that possibly one or more cofactors of the respiratory process become deficient. In view of our present knowledge of the coupling of oxidation to phosphorylation, the question arose as to whether adenylate or an equivalent phosphate acceptor might be limiting in the preclimacteric stage and subsequently formed with the rise of respiration to the climacteric peak. The immediate objective was therefore to determine the relationship between oxidation and phosphorylation in the course of the climacteric. In this paper attention will be focused first on experiments dealing with the metabolic reactions of the cytoplasmic particles, and subsequently studies will be cited on the trends of these reactions during the ripening process.

The method of isolation and the cofactor requirements for oxidation and phosphorylation were established in the case of the Fuerte avocado, a hybrid of *Persea americana* x *Persea drymifolia*. In order to maintain the metabolic integrity of these particles, the fruit tissue was subjected to low speed blending for one minute in hypertonic sucrose. The resultant homogenate was centrifuged at 500 x g to remove unbroken cells, large fragments, and nuclear material. The supernatant centrifuged at 17000 x g gave a precipitate which was suspended in a small volume of 0.5 M sucrose. All operations up to this point were carried out at 0°C. The final suspension containing 1 to 2 mg protein nitrogen per 0.5 ml was used in Warburg vessels for metabolic studies at 20°C. All oxidation reactions were carried out in 0.01 M phosphate, pH 7.1, 0.5 M sucrose, 0.02 M substrate, 0.001 M AMP, and 0.006 M $MgSO_4$. Addition of cytochrome c was not necessary for the oxidation of any of the acids of the Krebs cycle. Requirement for adenylate became marked when the reaction progressed beyond the first hour. Constant rates of oxydation for two to three hours were found for most of the acids of the Krebs cycle. The QO_2 (N) values ranged from 60 for pyruvate to 400 for succinate.

The cytoplasmic particles of the avocado exhibited also a high rate or phosphorylation. Phosphate uptake was markedly increased by the addition of catalytic quantities of adenylate, and by glucose. Apparently a particle bound hexokinase brought about the esterification of glucose. The coupling of phosphorylation to oxidation was expressed by P/O, the ratio of the microatoms of inorganic phosphorus disappeared from the reaction medium to microatoms of oxygen taken up by the suspension of the particles. P/O ratios of one to two were obtained for α-ketogluterate, malate, citrate, and pyruvate, and one for succinate. When the oxidation of α-ketogluterate was blocked at the succinate stage by the addition of 0.01 M malonate, the P/O ratio was higher than two. It was almost 3.0 if corrections were made for dephosphorylation by the enzyme ATP-ase.

The remarkable constancy and high metabolic activities of the cytoplasmic particles of the avocado made it possible to follow oxidative phosphorylation in this fruit in relation to the ripening process. The climacteric pattern and the factors affecting it have been worked out previously for the Fuerte variety of avocado. The fruits were placed in containers individually at 20°C, and air at a constant rate passed through these containers and into the Beckman oxygen analyzer. From the record of the oxygen uptake the position of the fruit in the climacteric cycle could be determined prior to blending. Studies with particles have shown that the oxidative capacity was not limiting at the preclimacteric minimum. With succinate the values were about the same in the ripe as in the unripe fruit. On the other hand, when α-ketogluterate, malate and pyruvate were oxidized the rates were lower at the peak. The phosphate uptake was high throughout the course of the climacteric.

The story was drastically changed when 10^{-5} M dinitrophenol (DNP) was added to the reaction medium. This widely accepted uncoupling agent was used because it was found to accelerate markedly the respiration rate of avocado slices at the preclimacteric stage, but not at the peak. In the case of the particles oxidation was not but phosphorylation definitely was affected by DNP. This reagent markedly lowered the P/O ratio in fruit fresh from the tree or at the preclimacteric minimum. At the climacteric peak the effect of DNP was reduced with some acids and abolished with others. These results suggest that the ripening process is in some way controlled by the phosphorylating mechanism of the cell. The full meaning of the uncoupling experiments might be comprehended better when we know more about the nature of DNP action.

THE EFFECT OF ETHYLENE ON THE RATE OF RESPIRATION OF APPLES, AS A FUNCTION OF TEMPERATURE

by J. C. Fidler

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The form of the graph for the rate of production of carbon dioxide by apples (and many other fruits) after picking is well known ; it is characterised by the occurrence of what is known as the « climacteric rise » (Kidd and West, 1921). At 20°C the interval between picking the fruit and the onset of the climacteric is short, and the climacteric is sharply defined ; as the temperature is lowered the interval lengthens and it becomes increasingly difficult to define the time of onset of the climacteric, or the peak value of the rate of respiration (Kidd and West, 1922).

The climacteric rise is accompanied by the production of ethylene (Gane, 1933) and the onset of ripening. Ripening and the climacteric can be prematurely induced by administering ethylene in low concentration in air (Harvey ; Denny ; Kidd and West).

These facts are important in the storage of many fruits. Thus bananas, which are carried on board ship at about 12°C, can be prematurely ripened by the presence in the cargo of pockets of ripe bananas. By analogy the argument has been extended to apply to apples and pears in refrigerated storage at 0° or 3°C.

To what extent is this contention justified ? Kidd and West (1934) attempted to test the effect of ethylene on apples at 3°C (at which temperature the rate of respiration is very low, and the climacteric not easy to detect). Their experiments were not conclusive, and they stated that « no direct trial has yet been made of the effect of ethylene on pre-climacteric fruit at low temperatures ».

Fidler (1948, 1950, 1951) has reported that apples and pears in gas-storage at 3°C or at 1°C begin to produce ethylene after about 20 days, and thereafter produce it at rates which keep the level of concentration up to about 100 parts per million ; the peak recorded value is 600 p.p.m. Apples and pears keep very much longer in gas storage than in air ; some months after fruit from refrigerated air stores has been marketed, the fruit from gas stores is still hard and green. Thus the effect of very high concentrations of ethylene over a long period is completely outweighed by the effects of changing concentration of carbon dioxide and oxygen. The apples taken from gas storage no longer show a climacteric in air. (The results for pears are more complex, and the analysis is not yet complete). Thus the coincidence between the climacteric, the production of ethylene, and the onset of ripening which holds at higher temperatures does not hold for the special case of apples or pears in gas storage at 3°C.

Apples in air at 3°C produce ethylene during the climacteric rise, and in this sense behave similarly to apples at 15°C, but two seasons work seems to show that their respiration is not sensitive to ethylene at the lower temperature.

In mid September 1952, two containers, each holding 5 kg. of freshly picked Edward VII apples were put at 3°C and ventilated at a rapid rate with fresh air drawn from outside the building. One part of ethylene to each 700 parts of air was added to the air stream entering one container. Special precautions were taken to keep the rate of flow of gas constant, so as to eliminate as far as possible irregularities in the readings of respiratory rate caused by alteration in the level of concentration of CO_2 in the containers. At the beginning of the experiment both lots of apples were pre-climacteric, and the rate of respiration declined from 55 mg. CO_2/10 Kg./Hr. to 40 in the first 22 days. Thereafter the rate rose steadily to a peak value of 60 on the 110th day. The two graphs, for apples in air alone and in air plus ethylene, were identical. At the end of the experiment, after 120 days, the two lots were indistinguishable.

The experiments were repeated in 1953, at 3°C, 7°C, and 12.5°C, and extended to include the effect of putting the pre-climacteric Edward VII apples in the same container as post-climacteric Worcester Pearmain apples. At 3°C the untreated apples began the climacteric rise after about 10 to 15 days ; those treated with ethylene or stored with Worcesters were a little later, about the 15th to 20th day. Thereafter the graphs were similar in level.

At 7°C, the climacteric in the control sample began on the 8th day ; in the two treated samples it began about 4 days earlier ; all three samples reached the peak value together on the 50th day.

At 12.5°C, the two treated samples began the climacteric rise immediately, but in the control sample it did not begin until the 5th day. All samples reached the peak value on the 20th day.

Also in 1953, a precisely similar experiment, begun two weeks earlier (but lasting only two weeks), gave the same result.

Thus, for this variety of apple at least, neither ethylene nor the vapour from ripe apples had any effect on respiratory activity at 3°C. At 7°C the effect was slight, and at 12.5°C it was marked.

This result may affect our way of thinking about the problems of storage at 3°C or lower ; it may mean some relaxation of the virtual ban on storage of mixed varieties. It must be emphasised, however, that ethylene has other undesirable effects, and the action of ethylene (or some other constituent of the apple « volatiles ») in producing lenticel spots or other skin lesions should not be forgotten.

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ORGANIC ACIDS IN THE APPLE FRUIT

by A. C. Hulme

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My remarks will be confined almost entirely to the organic acids which have only recently been found in the apple fruit and to brief speculations concerning the part they may play in the metabolism of the fruit.

Quinic acid : It was not until modern techniques of chromatography were used in a systematic survey of the acids of the apple fruit that the importance of this acid came to be recognised, Hulme (1951). In addition to the isolation of 1-quinic acid from the apple, Hulme and Swain (1951) and more recently in Australia Anet and Reynolds (1953) detected the acid in plums and peaches and Hulme and Richardson (1954) have isolated it from grass.

When apple fruits (Bramley's Seedlings, Worcester Pearmain and Cox's Orange Pippins have been examined) are at an early stage of development (∽ 14 g. fresh weight), quinic acid may account for more than half the total acid content of the *cortical* tissue. As the fruit matures the concentration and amount of this acid decreases rapidly.

The form of the curves in Fig. 1 suggests — as might be expected — that quinic acid and malic acid are concerned in quite different metabolic processes.

In the *epidermal* tissue (peel) of immature sweet apples the concentration of quinic acid also exceeds that of malic acid, and it remains high and may even approach the malic acid content in the mature fruit.

Interest in the occurrence of free quinic acid has been heightened by the isolation in considerable quantity also from apple fruits (HULME, 1953) of *chlorogenic acid* which is a depside of quinic and caffeic acids. As will be seen from Fig. 2, the o-dihydroxyphenolic groups of caffeic acid are free in chlorogenic acid, and consequently, it may very well serve as a substrate for the polyphenolase present in the fruit.

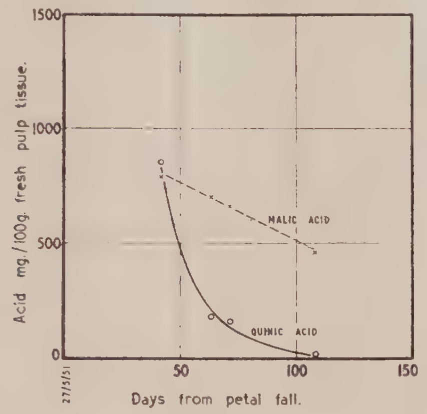


Fig. 1. — Changes in malic and quinic acids in the cortical (pulp) tissue of Worcester Pearmain apples during development on the tree.

It is interesting to compare the amounts of quinic and chlorogenic acids present in the pulp and peel tissue of various apples at different stages of

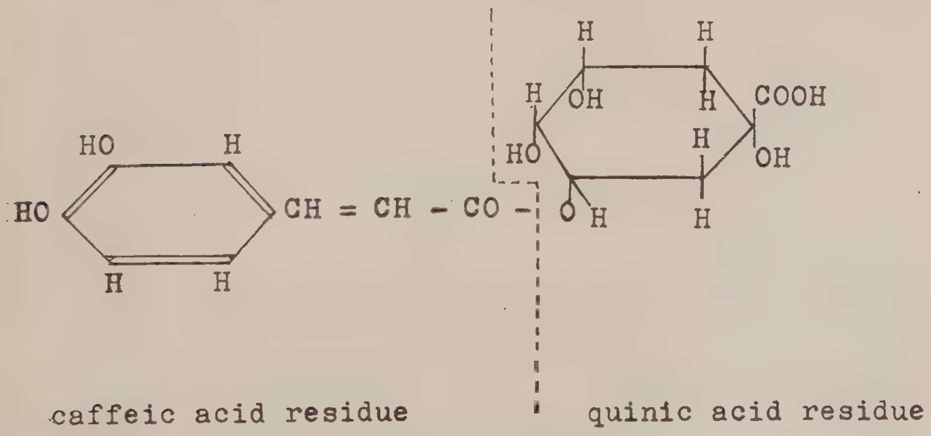
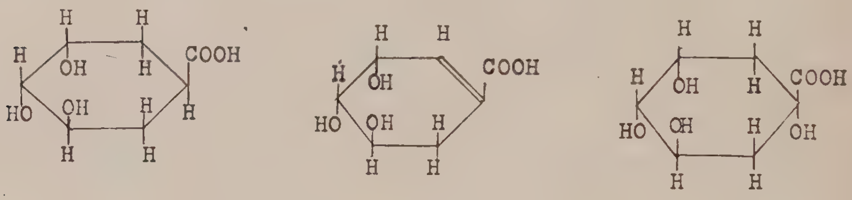


Fig. 2. — Formula of Chlorogenic Acid.

development. Although the figures given are only approximate they serve to demonstrate a relationship between the two acids present from tissue to tissue. PHILLIPS at Long Ashton has shown that in the juices of cider varieties high quinic acid is accompanied by high chlorogenic acid content. It is possible that one role of quinic acid is, by combining with the relatively insoluble caffeic acid, to render the o-dihydroxyphenolic properties of this latter acid more readily available in the form of chlorogenic acid. Quinic acid would then be analogous to the sugar moiety of the anthocyanins.

It has also been suggested that quinic acid and the related acids dihydroshikimic and shikimic might form part of a dehydrogenase system analogous to the succinic-fumaric-malic system as shown in Fig. 3.



dihydroshikimic acid ± 2H $\rightleftharpoons$ shikimic acid ± H_2O $\rightleftharpoons$ quinic acid

Fig. 3. — Postulated relationships between quinic acid, shikimic acid and dihydroshikimic acid.

It is perhaps, significant that the quinic acid content is relatively high in young apples and peel tissues which have a high level of metabolic activity, and a relatively high protein (enzyme) content.

Recently another acid — and one which has not apparently previously been reported in plant or animal tissues — has been found in the peel of both young and mature apples. This acid which was at first thought to be β-hydroxyglutaric acid has now been isolated and identified as the isomeric *citramalic acid* (1-methyl malic). The confusion arose because the two isomers have almost the same melting point, have very similar R_F values in at least two solvents and both yield the 2.4.dinitrophenylhydrazone of acetoacetic acid on oxidation and coupling with dinitrophenylhydrazine (HULME, 1954). This acid was first noticed on paper chromatograms of extracts of whole apples (excluding seeds) of mature Edward VII apples. Subsequently it was tracked down to the peel. By the use of ion exchange resins, fractions were obtained from extracts of the peel which contained a mixture of malic acid and citramalic acid (HULME, 1954). Subsequent work has shown that this acid is absent from the cortical tissue of apple fruits, is more abundant in the peel of mature than young fruits, and varies considerably in amount with apple variety. In mature fruits, Worcester Pearmain peel contains more citramalic than malic, Bramley's Seedling peel contains rather less citramalic than malic, and Cox's Orange Pippin peel contains only a very small amount of citramalic. Citramalic appears to decrease during the storage of the fruit.

The known facts regarding citramalic acid are that (1) it is confined to the « wax » producing peel tissue, (2) it is more abundant in mature fruits which contain more wax than immature fruits, (3) *in vitro* it is readily oxidised (H_2O_2 or Fenton's reagent) to aceto-acetic acid, an intermediate in fat metabolism. Aceto-acetic acid readily decomposes to acetone which is found in relatively large amount in the volatile substances given off by mature fruits.

Remarks on the evidence for the existence of a Krebs cycle mechanism in the apple fruit. — Millerd, Bonner and Biale (1953) have provided evidence for the existence of a cyclophorase system in the avocado and I believe Prof. Biale has more to say on this subject at the present Congress.

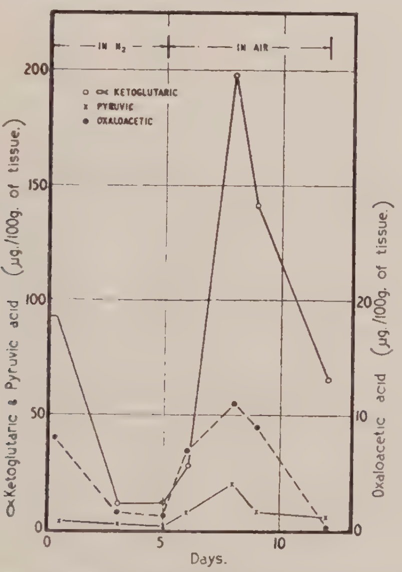


Fig. 4. — Changes in the α-keto glutaric, pyruvic and oxaloacetic acids content of the whole tissue (excluding seeds) of Bramley's Seedling apples during storage at 15° C. in nitrogen and after subsequent transfer to air at the same temperature.

The isolation of individual enzymes or particulate enzyme systems from tissue with so low a total-enzyme content and « bathed » by such an acid sap as that of the apple is not easy. We may, however, consider what Krebs cycle acids are present in the apple and how they vary with conditions.

In mature fruits but not in immature ones we have found traces of citric acid (and, incidentally, in the *peel* citric and oxalic acids), but of greater interest is the detection as their dinitrophenylhydrazones of pyruvic, α ketoglutaric and oxaloacetic acids (small amounts of glyoxylic acid have also been detected by this method). There is evidence also that these acids, and especially α ketoglutaric acid decrease in the absence of oxygen with, presumably, an accumulation of their precursors since, on readmission of air, there is a rapid and temporary burst in their rate of production (see Fig. 4).

NITROGEN METABOLISM OF THE APPLE FRUIT WITH SPECIAL REFERENCE TO THE RESPIRATION-CLIMACTERIC

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In the mature fruit the protein content, which seldom accounts for less than 50 % of the total nitrogen present, is so low that there are only 2-3 atoms of nitrogen per square Angström unit of cell surface of the apple as a whole. Throughout the life of the fruit, the greater proportion of the total N can be accounted for as protein and amino acids — of the amino acids, asparagine predominates. The apple is very poor in the basic amino acids and we have not so far detected any of the sulphur containing acids free in Bramley's Seedling, Worcester Pearmain or Cox's Orange Pippin apples. The amino acid pattern of hydrolysates of crude protein preparations from the apple is very similar to the pattern of the free amino acids with the expected differences that in the hydrolysates only the α-amino acids are present, hydroxyproline is absent and arginine and lysine appear in small amounts (Fig. 1). In Fig. 1, lysine is absent but this acid (as well as some of the arginine) was retained by the ion exchange resin from which the other acids were displaced by 0.1 N ammonia and was subsequently found in the HCl eluates from the resin (Hulme, 1951).

The amounts of α-alanine present in all the apple tissues examined are relatively large. Since aspartic acid is always present in fair quantity and pyruvic acid and oxaloacetic acid are also present, it appears possible that this α alanine may arise directly by transamination from aspartic acid without the usual mediation of glutamic acid.

In tissue having a relatively high metabolic activity, large amounts of γ amino butyric acid and very appreciable amounts of the newly discovered amino acid piperidine 2-carboxylic acid are present. In mature pulp tissue and also in the juice from mature apples (Burroughs, 1933) γ amino butyric acid is present in much smaller amount and may even be absent and piperidine-2-carboxylic acid is also present only in trace, if at all. Finally the number of imino acids present in the apple is striking ; apart from proline and hydroxy-proline, a methyl proline which is probably γ methyl proline has been isolated in small amount from young Worcester Pearmain apples. McKee and Urbach have found an acid related to proline which they suggest may be 4-hydroxymethyl-proline in the Granny Smith apple. Piperidine-2-carboxylic acid has already been mentioned, and very recently in my laboratory we have detected the presence in relatively large amount in the peel of young apples of an acid which from its position on 2-dimensional chromatograms and its colour reactions with isatin and ninhydrin contains either a pyrrolidine or piperidine ring.

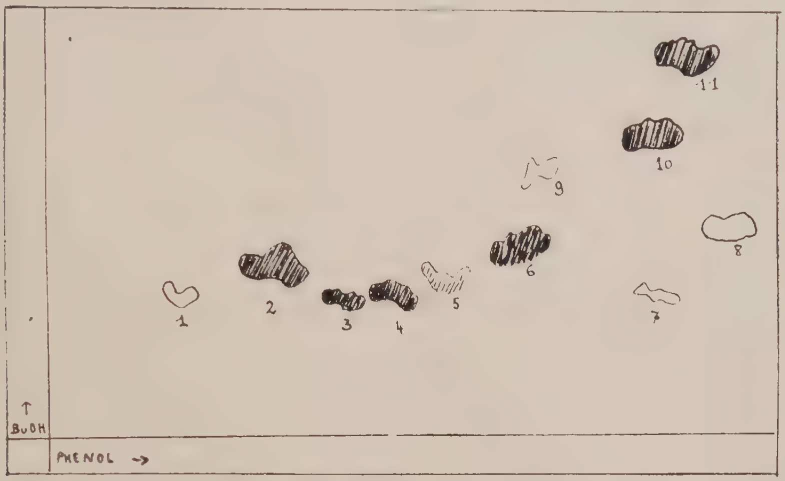

Fig. 1. — Paper chromatogram from hydrolysate of an apple protein fraction. (Bramley's Seedling). 1 : aspartic acid ; 2 : glutamic acid ; 3 : serine ; 4 : glycine ; 5 : thréonine ; 6 : α -alanine ; 7 : arginine ; 8 : proline ; 9 : tyrosine ; 10 : valine ; 11 : leucine.

A striking parallelism exists between the development of the climacteric and change in P. E. (non-protein nitrogen) in Bramley's Seedling fruits detached from the tree at various stages of development and stored at 12°C.

In fruit picked before cell division has ceased (24 days from petal fall) carbohydrate (and possibly phosphate) reserves are low and synthetic processes are limited. This fruit never develops a climacteric and there is never an increase in net protein. Fruit picked later develops a full climacteric and a net increase in protein N amounting to 10-15 % of the total N occurs at about the same time. In the earlier of these picks developing a « full » climacteric, rise in protein *precedes* the climacteric rise, the two rises tending to occur simultaneously in mature fruit. In these earlier picks synthesis of sucrose is maintained for some time after picking and *before* the fall in sucrose gets under way, increased synthesis of protein commences providing an added sink for energy rich phosphates. This leads to the rapid onset of the respiration climacteric. Subsequent fall in sucrose is more than offset by an increasing rate of protein synthesis and respiration continues to increase, the climacteric peak being reached as net increase in protein falls off. Briefly, it is suggested that at least one factor contributing to the onset of the respiration climacteric is an increased protein synthesis leading to a reduction in the ATP/ADP ratio.

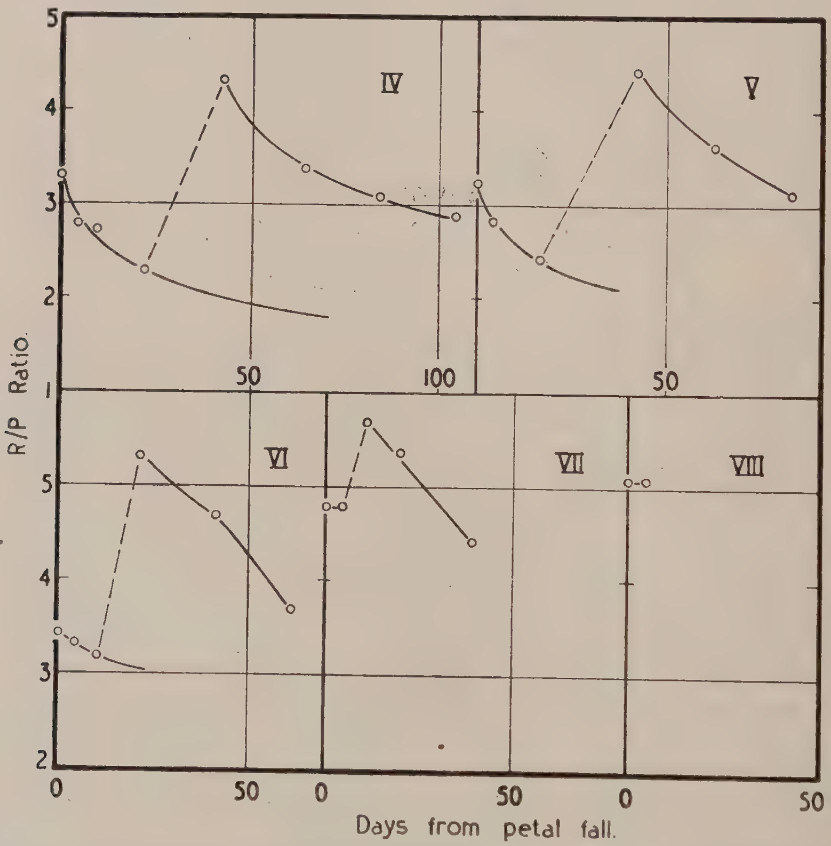

Fig. 2. — Changes in the rate of respiration per unit of protein (the R/P ratio) in Bramley's Seedling after picking at various stages of development. Season 1939.
Pick IV : 22 Aug. : 92 days from petal-fall.
Pick V : 13 Sep. : 114 days from petal-fall.
Pick VI : 4 Oct. : 135 days from petal-fall.
Pick VII : 26 Oct. : 157 days from petal-fall.
Pick VIII : 2 Nov. : 164 days from petal-fall.

Finally, it should be made clear that although net protein synthesis increases over the climacteric period it does not increase to such a great extent as respiration *i. e.* the respiration/protein ratio increases. In young fruit where no climacteric occurs there is no rise in the ratio. It will be seen from Fig. 2 that immediately on picking the R/P ratio falls. Assuming that protein represents total enzymes, this fall may be due to a falling off in carbohydrate substrate. The rise in the R/P ratio at the climacteric (when protein is, nevertheless, also increasing) may be because the « new » protein being synthesised contains a higher proportion of the enzymes directly involved in respiration. This would provide an explanation for the continuance of respiration at a high, though falling, rate for some time after the peak. The post-climacteric fall in R might then be considered as reproducing the fall in the R/P ratio after picking and as being a result of limiting substrate.

Millerd, Bonner and Biale (1953) suggest that the climacteric in the avocado fruit results from a decrease in the degree of dependence of respiratory oxidation on coupled phosphorylation. It is not stated whether a net increase in protein accompanies the respiration climacteric in the avocado. One factor to which these workers suggest an uncoupling effect might be due is an ATP-ase. Possibly the change in the balance of enzymes in the « new » protein formed in the region of the climacteric as mentioned earlier may involve an increase in ATP-ase.

SOLUBLE NITROGENOUS COMPOUNDS IN DEVELOPING APPLE FRUITS

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The physiology of the developing Granny Smith apple fruit has been studied with special reference to cell enlargement by Bain and Robertson (1951), Robertson and Turner (1951) and Pearson and Robertson (1953).

McKee and Urbach (1953) studied the ninhydrin-reacting nitrogenous components of Granny Smith apple fruits at various stages of development over two seasons. In each season the samples included fruit in the early stages of development and also over-mature fruit left on the tree after commercial maturity.

Fresh material was extracted with ethanol. The ethanolic solution was passed down a column of « Zeo-Karb » 215, which retained amino-acids while allowing sugars to pass through. This separation of amino-acids from sugars greatly facilitated the subsequent paper chromatography of the amino-acids. The amino-acids were eluted from the column with ammonium hydroxide ; arginine, the only amino-acid which the column retained against ammonium hydroxide, was eluted with sodium hydroxide. The ammonium hydroxide eluate was concentrated and examined by twodimensional paper chromatography, the solvents most used being phenol followed by a mixture (4:1:1 by volume) of butanol, acetic acid and water (REED, 1950). The chromatograms were sprayed regularly with ninhydrin and with isatin ; other reagents were used for special tests.

Asparagine, though it crystallized out during the concentration of the ethanolic extracts, was always more conspicuous on the chromatograms than any other amino-acid or amide. The other prominent amino-acids were aspartic acid, glutamic acid, serine, threonine and alanine. Valine, proline, γ-aminobutyric acid, β-alanine, histidine, pipecolinic acid, methionine sulphoxide and « leucine » (either or both of leucine and isoleucine, which were not separated by the method used) were present as minor constituents throughout. Methionine sulphoxide may have been formed by the oxidation of methionine during analysis, as may methionine sulphone, which was detected sporadically. Arginine, glutamine, tyrosine and phenylalanine were found in some samples but not in all. Glycine could not be detected as a free amino-acid, though it was present in a hydrolysate of protein prepared from the same fruit as provided one sample of ethanol-soluble amino-acids, and was found by HULME and ARTHINGTON (1950) in apples of the varieties Bramley's Seedling, Cox's Orange Pippin and Worcester Pearmain. It may be noted that glycine is absent from the list of 13 amino-acids detected by MATSUDA and ASO (1952) in extracts of apple seeds. All the extracts of apple fruits and leaves examined contained a compound whose colour reactions with ninhydrin, isatin, and nitroprusside plus acetaldehyde, suggested a relationship with proline. On two-dimensional chromatograms, however, it separated from both proline and hydroxyproline. This compound has been isolated and its examination is in progress (URBACH, unpublished). The results so far available suggest that it may be 4-hydroxymethylproline.

The almost complete constancy of the amino-acid pattern during the development of the apple fruit was a striking and rather surprising feature of the results in each season. The concentration of individual amino-acids was high in the early stages, and then remained at a steady, comparatively low level until after commercial maturity, when it rose again. Glutamine was prominent in early and late samples, and gave only a faint spot in most intermediate samples. At 138 days after blossoming it could not be detected, even in extracts more concentrated than those usually used for chromatography. Glutamine disappears about the same time as starch accumulation reaches its maximum (PEARSON and ROBERTSON, 1953), suggesting a possible connection between the metabolism of protein and of carbohydrate. If the views of ROBERTSON and TURNER (1951) are correct, the reduced glutamine content may reflect a shortage of adenosinetriphosphate, known (ELLIOTT, 1948 ; SPECK, 1949) to be required for its synthesis *in vitro*. The disappearance of glutamine is not due to any shortage of glutamic acid for its synthesis, as this amino-acid is prominent throughout. During the period of intense protein synthesis the amino-acids likely to occur as residues in protein molecules seem to be present in excess of the requirements.

It is still not clear which compounds are mainly involved in the increase of soluble nitrogen per cell in fruit left on the tree after commercial maturity. Analyses in one season showed an increase of soluble nitrogen per cell from 10 × 10^{-10}g at 160 days after blossoming to 24 × 10^{-10}g at 244 days. Amides increased from 8 × 10^{-10}g to 14 × 10^{-10}g, leaving a five-fold increase, from 2 × 10^{-10}g to 10 × 10^{-10}g for other soluble nitrogenous compounds. No quantitative results are available for total amino nitrogen, but the appearance of the chromatograms hardly suggested so great an increase. It is thus possible that compounds not reacting with ninhydrin may be involved.

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FORMATION DES CAROTENOIDES CHEZ LES VEGETAUX

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Tout se passe, chez les Végétaux, comme si les multiples caroténoïdes qui ont été trouvés dans leurs tissus dérivaient de trois molécules fondamentales seulement :

1°) L'acétone et l'éthylène, qui, en se condensant avec perte d'une molécule d'eau, engendrent l'isoprène C_5H_8.

2°) L'acétone et l'acétaldéhyde, qui, en se condensant avec perte de la fonction alcool tertiaire, par déshydratation, engendrent l'aldéhyde β méthylcrotonique C_5H_8O, ou son isomère l'isoprénol.

La condensation de 8-i molécules d'isoprène, avec i molécules d'isoprénol (i = 4, 5, 6, 7, 8), engendrera les caroténoïdes $C_{40}H_{56}O_n$, où n varie de 0 à 4, avec élimination de 4 H_2O.

La formation des caroténoïdes s'explique ainsi par de simples réactions de condensation et de déshydratation, sans faire appel ni à des réactions d'oxydation, ni à des réactions de réduction.

Il est très remarquable que les caroténoïdes des tissus végétaux peuvent se partager chimiquement et physiologiquement en deux groupes :

1. Les caroténoïdes des organes massifs : fruits, racines, graines. Ces caroténoïdes sont constitués principalement par des carbures : lycopène, carotènes, γ, β, α (dans l'ordre où ils dérivent du lycopène).

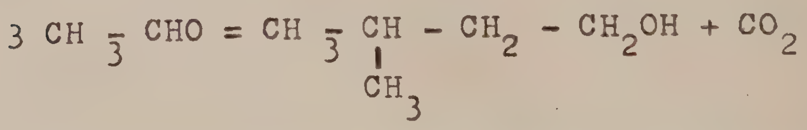
Lycopène et carotènes dérivent de la condensation de 4 molécules d'isoprène avec 4 d'isoprénol, et élimination de 4 H_2O. Parfois existent des carbures en C_{40} H_{60} et C_{40} H_{64}, peu colorés (carotène dzéta), dérivant de 6 isoprène + 2 isoprénol, ou dérivant de 8 isoprènes.

Ces caroténoïdes des organes massifs, où prédominent les carbures, peuvent donc être considérés comme dérivant principalement de l'éthylène. L'éthylène se dégage précisément de beaucoup de fruits au moment de la maturation, et au moment de la formation des caroténoïdes.

2. Les caroténoïdes des organes minces, feuilles, sépales, pétales ; ici prédominent quantitativement les alcools $C_{40}H_{56}O_2$, $C_{40}H_{56}$ O_4. Dans les feuilles coexistent des carbures et des dialcools ; dans les pétales on trouve des di et tétralcools, parfois des tétralcools seuls. Dans les algues brunes se rencontrent des caroténoïdes bruns qui sont à 6 fonctions alcool.

Ces caroténoïdes des organes minces dérivent donc principalement de l'acétaldéhyde, par l'intermédiaire de l'isoprénol. L'étude détaillée de la réaction de condensation permet de prévoir que les deux premières fonctions alcools seront en 3 et 3', ce qui a été vérifié pour les sept caroténoïdes mono ou dialcools décrits.

La formation de caroténoïdes existe chez beaucoup de levures. L'alcool amylique de fermentation peut être considéré comme le produit de réduction de l'isoprénol ; il ne dérive pas des aminoacides, mais du sucre par les intermédiaires : acide acétique, acétone, acétaldéhyde, qui subissent un début de condensation isoprénique, et sont réduits au cours de la fermentation. L'équation suivante rend compte du bilan de la formation d'alcool amylique :



$$3\ CH_3-CHO = CH_3-\underset{\underset{\displaystyle CH_3}{|}}{CH}-CH_2-CH_2OH+CO_2$$

Les bilans quantitatifs de fermentation alcoolique sont d'accord avec cette équation.

GAS EXCHANGE IN GRANNY SMITH APPLES

by E. G. HALL, F. E. HUELIN, Frances M. V. HACKNEYS° and Joan M. BAIN

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The composition of the atmosphere in the intercellular spaces of the flesh of the apple varies considerably with age of the fruit and is greatly affected by temperature and the composition of the external atmosphere. It can be extensively modified by the application of artificial skin coatings. Considerable differences in both oxygen and carbon dioxide tension between the internal and external atmospheres may appear during the life of the fruit indicating the development of considerable resistance to the exchange of these gases. Although there may be small gradients in gas concentration across the flesh this resistance is in the skin and probably mainly in the cuticle. As in mature Granny Smith apples the lenticels are non-functional and there is normally no passage from the core cavity to the calyx, gas exchange takes place by diffusion through the skin. The increase in resistance with age is due principally to the « waxing up » of the skin ; in the Granny Smith variety the greatest increase is in the oil content of the cuticle. Gas exchange through the skin probably takes place by movement in gas phases, in aqueous solution and in solution in the lipids of the cuticle. Resistance is little affected by temperature. The rate of gas exchange is controlled by the resistance of the skin to the movement of oxygen and carbon dioxide and by the rate of respiration oxygen consumption and carbon dioxide production by the tissues. As is consistent with its lower solubility in lipids, the resistance to oxygen is greater than that of carbon dioxide.

THE STRUCTURE OF THE MATURE APPLE FRUIT IN RELATION TO GASEOUS EXCHANGE

by W. Hugh SMITH

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The relation between structure and gaseous exchange in the apple fruit is of considerable interest in view of the effect of the composition of the external atmosphere upon the metabolism of the detached fruit (1). The

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apple fruit is composed largely of parenchymatous tissue bounded by a skin consisting of a hypodermis of flattened cells with thick cellulose walls and an epidermis composed of somewhat smaller cells with heavily cutinised outer tangential walls. This cutinised layer is covered by wax and is interrupted by lenticels, the number and form of which vary with variety of apple. The internal atmosphere of the fruit is contained by the locules and the spaces between the large parenchymatous cells of the flesh and is connected with the outer atmosphere through the lenticels. The volume of the internal atmosphere of the flesh has been shown to very between twenty and thirty five per cent of the total volume (2). Early maturing varieties have a more porous structure than later-maturing varieties.

The lenticels of the apple fruit are of three main types and are derived from the degenerated bases of hairs or from stomata (3). The larger lenticels take the form of breaks in the epidermis beneath which there is a crater-like depression with a layer of cork cells forming the « floor » which overlays the hypodermis. In « open » lenticels this floor is cracked and gives access to the internal atmosphere. Smaller lenticels consist of a minute break in the epidermis often formed by enlargement of the stomatal aperture. The underlying hypodermal cells may or may not be suberised.

Not all lenticels are open to mass movement of the atmosphere. It is a matter of some difficulty to determine with certainty which are open and which closed.

The numbers of lenticels falling into the classification just described were determined for 129 apples of the Bramley's Seedling variety drawn from various sources **(Table I).**

Table I

The Number and Distribution of Types of Lenticel in Bramley's Seedling Apples

Type of Lenticel	Mean Stem-end	Mean Calyx-end	Mean Total	Range
Large with suberised 'crater')	117	483	510	193 to 792
Small with suberised hypodermal) layer)			66	0 to 234
Small with no suberisation)			24	0 to 246
All kinds)			600	

There is a considerable variation in the number of lenticels occurring on individual fruit of the same variety. On an average there are more than four times as many lenticels on the calyx half of the fruit than on the stem half.

Exchange of water-vapour, nitrogen, oxygen, carbon dioxide, ethylene, alcohol, acetaldehyde, volatile esters and essential oils takes place between the outer and the inner atmosphere of the fruit. It has been concluded that only 8-25 % of the transpiration of apples is accounted for by passage of water vapour through the lenticels (4). The layer of wax on the cuticle is of great importance in diminishing water loss.

Nothing is known about the manner in which the volatile constituents of the fruit pass through the skin.

The way in which carbon dioxide diffuses out and oxygen into the fruit is still not fully understood. It seems probable that the primary path for carbon dioxide is through the open lenticel. Thus stoppage of lenticels with oily substance causes a temporary check to the rate of carbon dioxide evolution. Painting the skin surface between the lenticels only slightly checks carbon dioxide output.

The internal content of carbon dioxide increases steadily and the internal oxygen content declines with the progressing senescence of the fruit.

This is attributed to a change in porosity of the skin (5). This change in porosity can be shown to be associated with closure of lenticels. The closure is due to increased suberisation.

It has been observed that the sum of the internal carbon dioxide and oxygen concentration which is initially 21 % declines with the ageing of the fruit. This cannot be explained by difference in the rate of production of carbon dioxide and oxygen since during senescence the Respiratory Quotient for the apple is greater than unity. It seems therefore that resistance to the entry of oxygen is greater than the resistance to the escape of carbon dioxide and that it increases with time. It can moreover be shown that the pressure of the internal atmosphere is slightly lower than that of the external atmosphere.

The most likely explanation seems to be that carbon dioxide is able to take a path of diffusion through suberised tissues which is almost if not quite impermeable to oxygen. But experimental evidence to support this is still lacking.

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SECTION 12

BOTANIQUE AGRONOMIQUE

ETUDE DE LA NUTRITION DU CAFEIER PAR LA METHODE DU DIAGNOSTIC FOLIAIRE

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L'étude de la nutrition minérale du caféier a été commencée en Côte d'Ivoire en 1950, par la méthode du diagnostic foliaire, telle qu'elle a été définie par Lagatu et Maume le 13 juin 1928 dans les C. R. des séances de l'Académie d'Agriculture de France.

Cette première étude a permis :

1°) D'établir les courbes d'absorption de N, P_2O_5, K_2O, CaO par le caféier au cours de l'année.

2°) De suivre l'équilibre N/P/K et les rapports N/K, Ca/K, etc...

3°) D'établir des relations entre les rendements et la composition chimique de la feuille.

I. — *Le diagnostic foliaire.*

a) L'absorption d'azote par le caféier dans les conditions de la Station d'Akandjé est intense du début de mai au 15 juillet. La grande saison des pluies est le moment où la nutrition azotée est à son maximum. Elle baisse en août-septembre (petite saison sèche), mais dans des proportions restreintes, pour remonter assez facilement en octobre-novembre. Le second maximum de la nutrition azotée se situe au cours de la petite saison des pluies. La grande faim d'azote se précise dès le début d'avril et s'accentue en mai. La période hautement intéressante est celle du changement d'orientation des courbes. Il importe que le caféier trouve à sa disposition à ce moment de l'azote assez vite assimilable. Ce moment est variable, dans les conditions locales, avec la pluviométrie de l'année. Si la saison des pluies est tardive, c'est-à-dire si le mois de mai est plutôt sec, les caféiers souffrent du manque d'absorption d'azote et jaunissent fortement. On a ainsi pu indiquer les dates d'épandages des engrais azotés semblant les meilleures.

b) *L'absorption de phosphore.* — La nutrition phosphorée suit assez fidèlement la pluviométrie. Pendant la durée de l'essai, l'azote a fluctué de 2,06 3,10 % de la matière sèche, soit de 50 % : et P^2O^5 de 0,161 à 0,339, soit de plus de 100 %. La nutrition phosphorée est, en première approximation, intense du début de juin à décembre. Le caféier, dans les conditions locales, doit être bien alimenté en phosphore dès le mois de juin. La corrélation entre les rendements et les teneurs en P^2O^5 est élevée en juin, juillet, août.

c) *L'absorption de potassium.* — La nutrition potassique est également sous la dépendance étroite de la pluviométrie.

L'absorption augmente tous les ans en octobre-novembre (petite saison des pluies) et baisse en décembre-janvier, puis remonte, fait très important, bien avant l'établissement de la saison des pluies.

La courbe est, en gros, ascendante de février à juin et descendante de juillet à janvier, à part une phase ascendante plus faible en octobre-novembre.

d) *L'absorption du calcium.* — Le calcium apparaît comme un cation privilégié d'accumulation dans la feuille considérée et pour de vieux caféiers. Il y a dans cette feuille une remarquable opposition entre K et Ca, l'un augmentant lorsque l'autre baisse, et inversement. Par suite, la courbe de CaO est, en gros, descendante de février à juin et ascendante de juillet à janvier.

II. — *Influence de l'apport d'éléments fertilisants sur le chimisme foliaire.*

Deux échantillons de feuilles homologues prises sur deux carrés de caféiers présentant les mêmes conditions de milieu, mais soumis à différents apports d'éléments N, P, K, sont le siège de processus différents qui doivent être en corrélation avec la nature de ces apports.

a) *Au regard de l'azote*, une formule binaire PK tend à faire diminuer l'absorption d'azote par le caféier et une formule NP tend à l'accroître.

b) *Au regard du phosphore.* La formule NP tend à accroître dans de fortes proportions l'absorption de P^2O^5 et la formule NK à la diminuer dans des proportions non négligeables.

Les trois formules N, P, NP ont engendré une sous-absorption très caractéristique de K^2O. Une formule d'engrais NP, telle que celle apportée (20 g. N, 20 g. P^2O^5 par pied), restreint considérablement l'absorption de K^2O par le caféier. Les formules N et P provoquent cette sous-absorption à un moindre degré. Le même processus a été vérifié pour la phase de croissance.

c) D'autre part, vis-à-vis du potassium, les fumures K, NK, PK, NPK ont accru l'absorption de K_2O très notablement.

III. — *Liaison entre les rendements et l'analyse foliaire.*

a) Liaison entre les rendements et la nutrition azotée :

Par rapport aux rendements de 1950, on a trouvé les coefficients de corrélation suivants : 8 février + 0,17 ; 7 juin + 0,81 ; 12 juillet + 0,55 ; 17 août + 0,65 ; 23 septembre + 0,61 ; 23 octobre + 0,56.

Par rapport aux rendements de 1951, les corrélations ont été de : + 0,67 le 18 mai 1951 et + 0,73 le 22 juin 1951.

Pour 1952, il n'a pas été trouvé de relation nette. Cette année-là, les doses des parcelles ont été très groupées en mai-juin-juillet aux environs de 3,2 % (pour des raisons de taille), et ce pourcentage semble voisin de celui au-delà duquel les excédents d'azote dans la feuille sont sans profit pour le rendement futur.

Dans les conditions locales, c'est donc en mai-juin-juillet qu'on enregistre les plus fortes corrélations entre la nutrition azotée et les rendements. La grande faim d'azote est en relation directe avec les rendements enregistrés à la récolte suivante. Il est possible d'établir une méthode de prélèvements foliaires limités aux époques clés, ce qui aurait le grand avantage, en milieu d'étude tropical, de limiter le travail.

Il sera peut-être possible de déceler ainsi assez vite les déficiences en azote par des prélèvements limités.

Notons également qu'il y a une bonne corrélation entre les doses d'azote le 7 juin 1950 et les rendements de 1951 (+ 0,79).

b) Liaison entre les rendements et la nutrition phosphorée :

On a trouvé des corrélations positives assez nettes entre les teneurs de juillet-août 1950 et les rendements de 1950 (+ 0,77 le 17 août), de même entre les teneurs du 30 juillet 1951 et les rendements de 1951, et entre les teneurs des 16 juin et 24 juillet 1952 et les rendements de 1952.

c) Liaison entre les rendements et la nutrition potassique :

Les graphiques n'ont pas montré de relation très nette. En ce qui concerne le calcium, les graphiques tendraient à montrer l'existence d'une certaine corrélation positive dans le cas de cet essai entre rendement et teneur en calcium.

La corrélation semble meilleure encore dans la zone des rendements relativement élevés.

CONCLUSION

Si nous appelons « niveaux critiques » les teneurs en N, P^2O^5, K^2O % de matière sèche, au-dessus desquelles l'apport de ces éléments est sans effet, on obtient le tableau suivant :

	Déficience certaine	**Très bon rendement**	**Excès probable**
Azote N	1,8	3,0 à 3,3	3,3
Phosphore P_2O_5	0,18	0,30 à 0,35	0,35
Potassium K_2O	0,30	1,2 à 1,8	?

Pour l'azote, le niveau critique est aux environs de 3 %. Il semble bien que d'une part les zones dites de déficience certaine soient assez rares en Côte d'Ivoire et que, d'autre part, il en soit de même des zones supérieures au niveau critique supposé. Les sols de la région des sables côtiers se situent dans la zone où l'apport d'azote est le plus favorable.

Pour le phosphore, le niveau critique proposé est de 0,35 %. Ce niveau a été présenté par quelques points du territoire situés très en dehors du réseau de champs d'essai. Les points Akandjé-Bingerville sont nettement en dessous de ce niveau et Gagnoa et Daloa un peu plus favorisés.

Pour la potasse, le niveau critique est ici déterminé avec un certain flottement. On considère comme fournissant un bon rendement les caféiers dont la teneur en K_2O est de 1,2 à 1,8 %. Au-delà de 2,2 % (?), il semble bien qu'il y ait véritable accumulation de luxe. Là encore, c'est la zone côtière de Côte d'Ivoire qui est la plus défavorisée. Des teneurs de 0,5 en K_2O y sont très fréquentes. Par contre, à Gagnoa, Daloa, Man, les teneurs se rapprochent du niveau critique.

Il apparaît nécessaire d'étudier les 3 éléments basiques K, Ca, Mg. La constance relative du taux de cendres provient de la constance relative de la somme de ces trois éléments. Le caféier est très sensible aux antagonismes de ces éléments entre eux.

Aussi les recherches actuelles concernent-elles sur tous les échantillons les dosages de C, N, P, K, Ca, Mg et sur certains échantillons les dosages de Fe, Mn, Zn, B.

Enfin, le plus souvent possible, ces travaux sont accompagnés de l'étude des sols et le laboratoire de pédologie étudie en particulier la dynamique des sols des mailles principales du réseau des essais. Cette association des deux méthodes semble nécessaire si l'on juge que c'est le sol à caféier qui doit présenter un certain équilibre entre les éléments susceptibles de réaliser l'optimum alimentaire de l'arbre.

MISE AU POINT D'UN MILIEU DE SEMIS POUR LES GRAINES D'UNE ESPECE DE VANILLIER DONT ON N'OBTIENT PAS LA GERMINATION SUR LES MILIEUX DE CULTURE CLASSIQUES

par J.-P. Tonnier
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Les semences de *Vanilla fragrans* (Salish.) Ames (*V. planifolia* And.), de *V. Pompona* Scheide et des hybrides obtenus sur ces deux espèces utilisées comme pieds-mères, germent sur les solutions nutritives mises au point par Bouriquet à Madagascar et Knudson aux U.S.A.

Par contre, d'autres espèces, tant américaines que malgaches : *V. Claviculata* Sw., *V. madagascariensis* Rolfe, *V. Coursii* H. Perr., *V. Decaryana*

H. Perr., et les hybrides obtenus de ces géniteurs sauvages, ne germent pas dans ces conditions expérimentales.

Or, à Madagascar, les semences des espèces endémiques et celles de *V. fragrans* germent spontanément. Ces cas sont assez rares ; ils ont cependant été observés à plusieurs reprises et il a été constaté qu'ils se produisaient dans des conditions sensiblement analogues.

Des essais de semis ont été entrepris en avril 1953 avec *V. Madagascariensis*, espèce spontanée très commune à Madagascar. Les graines provenaient d'une gousse d'un an ; elles furent immergées dans l'eau de javel du commerce pendant sept jours et ensemencées sur une gamme de milieux ajustés à des pH différents.

Elles ont germé en trois mois sur la solution nutritive de Bouriquet, enrichie avec du lait de coco. La formule de ce milieu s'établit donc ainsi :

125 grammes de feuilles, tiges et racines adventives de *V. fragrans ;*

20 grammes de terre dans un litre d'eau porté à ébullition pendant 30 minutes.

Après filtration, on ajoute à cette décoction 20 grammes de glucose et 5 grammes de levure. A 900 cc de cette solution, on ajoute 100 cc de lait de coco et 125 grammes de gélose.

La faculté germinative de ces semences est beaucoup plus faible que celle de *V. Planifolia*. Par la suite, il a été observé que sur ce milieu l'évolution des sphérules est normale, bien que lente. Ce résultat permet d'espérer qu'il en sera de même pour les hybrides obtenus sur ce pied-mère.

NOUVEAUX ESSAIS SUR LA GERMINATION DES SEMENCES DE *VANILLA PLANIFOLIA* ANDR.

par J.-P. Tonnier

Les semences de *Vanilla fragans* (Salish.) Ames (= *V. planifolia* And.) sont assez volumineuses (m̄ diamètre maximum = 0,313 mm) ; elles sont pourvues d'un tégument séminal dur et leur embryon n'est pas différencié. Les cas de germination spontanée sont rares et les essais de semis en pleine terre se sont soldés par des échecs à une exception près (Bouriquet a obtenu de cette façon un Vanillier en 1939). On a donc recours à la méthode de semis découverte par Noël Bernard et l'ensemencement est fait sur la solution nutritive de Bouriquet ou sur celle de Knudson.

Le *Rhizoctonia repens* N. Bernard, champignon symbiotique du Vanillier, est inoculé dans les tubes de culture après la germination et son action sur les sphérules se traduit par un accroissement très sensible de leur vigueur. Par contre, il n'a jamais été observé que le champignon ait une influence quelconque sur la germination quand on l'inocule au moment des semis. A la sortie des tubes de culture, une partie du matériel végétal restant stationnaire sans raison apparente, il est permis de se demander si le changement de milieu et de conditions de végétation imposé aux plantules au moment du passage en milieu naturel, passage sans transition et en définitive assez brutal, de la solution nutritive aseptique au compost de repiquage non stérilisé, ne provoquait pas, en partie tout au moins, cet arrêt provisoire de croissance.

Si cette manière de voir est juste, la solution du problème réside sans doute dans l'ensemencement des graines sur un milieu non synthétique, dans des conditions aussi voisines que possible des conditions naturelles

On supprime ainsi l'opération du passage en milieu naturel et les aléas qu'elle comporte, puisque le matériel végétal se trouve d'emblée sur son substrat définitif.

On en revenait aux semis naturels ; or, cette technique expérimentée à Madagascar en 1936 avait été abandonnée peu après, au profit des méthodes de Noël BERNARD.

Cette question a été reprise avec des semences mûres de *Vanilla Planifolia* en juin 1953. Le jour de la récolte, les graines ont été dégraissées à l'alcool et au toluène puis décapées à l'eau de javel du commerce pendant 48 heures. Les semis ont été faits en boites de Pétri sur papier filtre, sable de rivière ou toile d'amiante et placés en étuve à 32°. Aucun de ces substrats ne fut contaminé par le *Rhizoctonia repens.*

Or, dans tous les cas, les graines ont germé normalement et il a été observé que les germinations étaient plus nombreuses et plus avancées sur sable de rivière recouvert d'une rondelle de papier filtre.

Par ailleurs, les boîtes de Pétri dans lesquelles on avait mis la quantité d'eau juste nécessaire à l'humidification de ce substrat donnaient les meilleurs résultats.

Dans ces conditions la levée des graines débute environ dix jours après le semis ; et des comptages effectués le 28e jour ont montré que la faculté germinative oscillait entre 72 et 79 %. La germination se déroule de la façon suivante : on constate d'abord la présence de mamelons et de petits poils raides, transparents. Ces excroissances sont alignées en demi-couronne sur le pourtour de la graine du côté de son petit bout ; il est facile de constater que la ligne de déhiscence est ouverte et que ces protubérances en émergent. Les poils s'allongent rapidement, ils peuvent atteindre deux ou trois fois le plus grand diamètre de la graine ; par la suite, les lèvres de la fente s'écartent davantage et livrent passage à l'extrémité apicale de la sphérule. Sur ce milieu le développement végétatif du Vanillier ne dépasse pas ce stade.

Il devient alors nécessaire de repiquer les sphérules sur un substrat plus riche en éléments nutritifs. On utilise à cette fin des boîtes de Roux à moitié remplies de sciure de bois blanc légèrement décomposé. Ce milieu bistérilisé à 134° a été contaminé trois semaines auparavant par le *Rhizoctonia repens* et il est totalement infesté. Dans ces conditions le développement des sphérules se poursuit normalement. On les repique tous les mois environ sur sciure de bois que l'on enrichit progressivement avec du terreau de bois. Des résultats analogues ont été obtenus avec la *Vanilla Tahitensis* J. W. Moore et ses variétés, Tiarei, Haapape et Potiti.

Ce procédé de semis sur milieux inertes non stérilisés a été retenu en raison de son faible prix de revient et de sa simplicité. Il présente en outre l'avantage de rendre possibles certains traitements qui n'étaient pas réalisables sur les solutions nutritives stérilisées (traitement aux hormones et aux substances mitoclasiques en particulier).

Les conséquences théoriques de cet essai ne sont pas moins importantes : on constate que le déclanchement des divisions cellulaires de la *V. Planifolia* n'est provoqué ni pár le symbiote ni par la forte concentration du milieu de semis puisque ces deux facteurs ne figuraient pas dans les essais décrits plus haut ; seuls les agents physiques *chaleur* et *humidité* sont donc entrés en ligne de compte.

Il en découle que les phénomènes de la germination ont un aspect très particulier chez ces espèces et qu'ils ne rappellent en rien ceux que l'on observe d'une façon très générale dans la famille des Orchidaceae.

Il semble que ce cas puisse être rapproché de celui de la *Bletilla hyacinthina* étudié par Noël Bernard. On sait que les semences de cette orchidée, pourvues d'une ébauche cotylédonaire, peuvent germer sur des milieux très dilués, avec ou sans champignon. La graine du Vanillier n'atteint certes pas ce degré de différenciation, mais elle est assez volumineuse et il est peut-être possible de rechercher dans ce caractère botanique la raison de cette singularité.

CARENCES MINERALES PRESENTEES PAR DES LEGUMINEUSES ET GRAMINEES FOURRAGERES DANS LA REGION DU LAC ALAOTRA (MADAGASCAR)

par J. Velly et P. Roche

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Au cours de l'année 1953, on a pu observer, à plusieurs reprises, des cas de troubles de la végétation chez diverses légumineuses et graminées fourragères. Ces troubles sont apparus le plus fréquemment sur des sols latéritiques rouges formés sur amphibolites. Certains des cas étudiés ont été également observés, soit sur alluvions fluviatiles latéritiques récentes, soit sur des fonds de vallées, formés de colluvions, ayant évolué en sols de marais et possédant une couche humifère importante en surface.

Pour tous les cas observés on a prélevé des feuilles présentant des symptômes de carence (feuilles naines, jaunes, brunes, rouges) et des feuilles d'apparence saine devant servir de comparaison. Ce matériel a été séché à l'étuve à 105°, broyé et analysé. Sur une partie de l'échantillon

(500 mgrs), on a dosé l'azote par la méthode de Kjeldahl. Sur une autre partie (250 mgrs) on a fait une attaque sulfo-nitro-perchlorique et l'on a dosé le phosphore par colorimétrie, le magnésium par colorimétrie à l'aide du jaune de titane, le potassium et le calcium au photomètre à flamme Beckman.

1° *Dolichos lablad.* Cette légumineuse était cultivée sur sol rouge latéritique.

Elément dosé	Feuilles malades	Feuilles saines
Azote %	3,76	4,52
Phosphore %	0,454	0,555
Potassium %	0,333	1,684
Calcium %	0,937	0,630
Magnésium %	0,115	0,288

Ce tableau montre une carence en potassium extrêmement nette chez les feuilles malades. Pour les autres éléments, les différences sont peu sensibles.

Des apports expérimentaux de chlorure de potassium à raison de 300 kg/ha supprimèrent totalement les symptômes de carence. Il apparut à l'analyse du sol que cette dose avait fait passer les teneurs en K_2O échangeable de 0,028 à 0,048 p. mille. On peut donc considérer la teneur en K_2O échangeable de 0,05 p. mille comme le seuil en dessous duquel apparaissent les phénomènes de carence en potassium.

2° *Mélange fourrager Vigna sinensis/Euchlaena mexicana.*

Ces fourrages, cultivés sur un marais récemment récupéré, présentaient deux symptômes. Chez les *Euchlaena,* des bandes rouges-brunes, situées en général au bord des feuilles. Chez les *Vigna,* les feuilles présentent des taches brunes, irrégulièrement réparties sur la surface foliaire.

L'analyse des feuilles saines et malades a donné les résultats suivants :

Nature de l'échantillon	Azote %	Phosphore %	Potassium %	Calcium %	Magnésium %
Vigna malades	3,96	0,164	1,666	1,826	0,720
Vigna sains	4,24	0,425	2,204	1,418	0,676
Euchlaena malades	3,16	0,141	1,556	0,680	0,444
Euchlaena sains	3,80	0,280	2,732	0,480	0,444

A l'examen de ce tableau on voit apparaître, chez les deux végétaux malades, une double carence en potasse et en phosphore.

Une analyse du sol a montré des teneurs en K_2O échangeable inférieures au seuil de 0,050 p. mille précédemment déterminé.

Quant à la carence en P_2O_5 elle s'est produite malgré un apport antérieur de 200 kg/ha de phosphate tricalcique. Quelques semaines après un apport supplémentaire de 200 kg/ha de phosphate tricalcique et de 250 kg/ha de chlorure de potasse, la végétation avait repris son aspect normal.

3° *Luzerne.* Cette légumineuse était cultivée sur sol rouge latéritique et les feuilles montraient des taches brunes qui caractérisent souvent la carence en potasse. Là aussi on a prélevé des feuilles malades et des feuilles saines et le résultat de leur analyse a montré qu'on se trouvait toujours en présence de la même carence en potasse et en phosphore beaucoup plus nette d'ailleurs dans le cas de la potasse. Là aussi l'application simultanée d'engrais phosphatés et potassiques a entraîné le retour à la végétation normale.

4° *Vigna sinensis en culture dérobée du riz.* Rizière sur alluvions fluviatiles latéritiques récentes. Après deux années de belle végétation (25 à 30 T. de matière verte à l'ha.) on a observé des taches brunes sur les feuilles de *Vigna* et une végétation irrégulière ; les résultats analytiques de ces feuilles sont les suivants :

Nature de l'échantillon	Azote %	Phosphore %	Potassium %	Calcium %
Vigna feuilles saines	3,24	0,238	1,675	0,877
Vigna feuilles malades	1,76	0,225	0,727	1,007

On peut noter, toujours aussi nettement, la carence en potasse des feuilles de *Vigna* malades. On peut observer aussi, comme dans tous les cas de carence en potasse cités jusqu'ici, un enrichissement simultané de la feuille malade en calcium.

Nous avons pu observer par ailleurs que l'apport de phosphate sur le riz se traduisait par une plus grande absorption de l'azote du sol qui peut manquer ensuite pour l'engrais vert, ce que laisse supposer le tableau ci-dessus.

5° *Euchlaena mexicana,* présentant des feuilles tachées de brun et des feuilles tachées de rouge, surtout aux extrémités et sur les bords. L'analyse de ces différentes catégories de feuilles donne les résultats suivants :

Nature de l'échantillon	Azote %	Phosphore %	Potassium %	Calcium %
Euchlaena feuilles saines	2,64	0,259	2,833	0,145
Euchlaena feuilles rouges	1,56	0,190	1,571	0,200
Euchlaena feuilles tachées	2,40	0,222	1,166	0,200

Comme dans les autres cas observés, l'apparition de taches brunes correspond à une carence potassique et les feuilles rouges sont en général pauvres en phosphore.

CONCLUSIONS. — Sur les sols rouges latéritiques de la Station de l'Alaotra la plupart des cultures fourragères et engrais verts poussent très mal, montrent des feuilles jaunies, déformées et tachées de brun. Leur analyse systématique a montré chez toutes une carence très nette en potasse sauf pour le Kudzu (*Pueraria javanica*). Par contre presque tous ces végétaux carencés en potassium présentaient des teneurs plus fortes en phosphore.

Ces différentes recherches ont permis de mettre en évidence la nécessité d'une fumure potassique sur les sols rouges latéritiques de la région du lac Alaotra. Actuellement, chaque fois que la teneur du sol en potasse échangeable est inférieure à 0,05 p. mille, il est indispensable d'apporter au moins 200 kilos de chlorure de potassium à l'hectare.

Ces mêmes sols étant le plus souvent également très pauvres en acide phosphorique assimilable, on peut apporter à la fois des phosphates et de la potasse. Dans tous les cas observés jusqu'ici, ce double apport a suffi pour redonner à la végétation son apparence normale.

TERRES SALINES ET VEGETATION DE CERTAINES MERDJAS DE LA PLAINE DU GHARB (MAROC)

par J. DE BOISSEZON

Grenoble

Dans le cadre de l'étude agronomique des 22.000 ha. du Domaine public en rive gauche du Sebou, l'étude de la végétation nous a permis d'observer l'influence de la salinité du sol sur les cultures, les espèces spontanées et les groupements végétaux.

Cette région de la plaine du Gharb est un exemple typique du « paysage de basse plaine alluviale » : les rivières circulent sur des « levées » de terres légères, au sommet desquelles est un lit profond, mais insuffisant en période de crue. En s'éloignant du fleuve, les pentes s'adoucissent lentement ; les sables font place aux limons, puis aux argiles qui occupent les « bassins » ; dans ces zones basses, à mauvais drainage, les eaux stagnent. Cette répartition des sédiments s'explique aisément par l'ordre de dépôt des particules pendant les inondations.

Dans ces conditions, les sols salins correspondent soit à la concentration des eaux superficielles dans les zones basses, soit à la remontée du sel de la nappe phréatique. La salinité de ces sols est de : 0,2 à 1 % (sels totaux) en surface et 0,6 à 1,5 % en profondeur.

L'action de la salinité superficielle et profonde sur les rendements des cultures est indiquée par le graphique (1). La culture n'est tentée que très rarement dès que la salinité superficielle dépasse 0,5 % (sels totaux), et de fortes dépressions de récoltes sont visibles pour l'orge à partir de 0,1 %.

La présence des différentes espèces végétales est indiquée par le graphique (2), qui donne pour les plantes les plus caractéristiques de la zone étudiée leur fréquence de présentation pour chaque classe de salinité du profil. Cette planche montre le caractère plus ou moins halophile de chaque espèce.

Mais l'influence fondamentale de la salinité sur la végétation se traduit en particulier par la formation de groupements végétaux qui indiquent assez bien les différents types de sols salés. Le tableau (3) indique schématiquement la correspondance entre les types de sols et les différents groupements végétaux.

Enfin un profil d'observation des bords d'une zone inondée illustre l'interaction de la salinité et des conditions hydriques.

FIGURE 1

INFLUENCE DE LA SALINITE
SUR LES RENDEMENTS

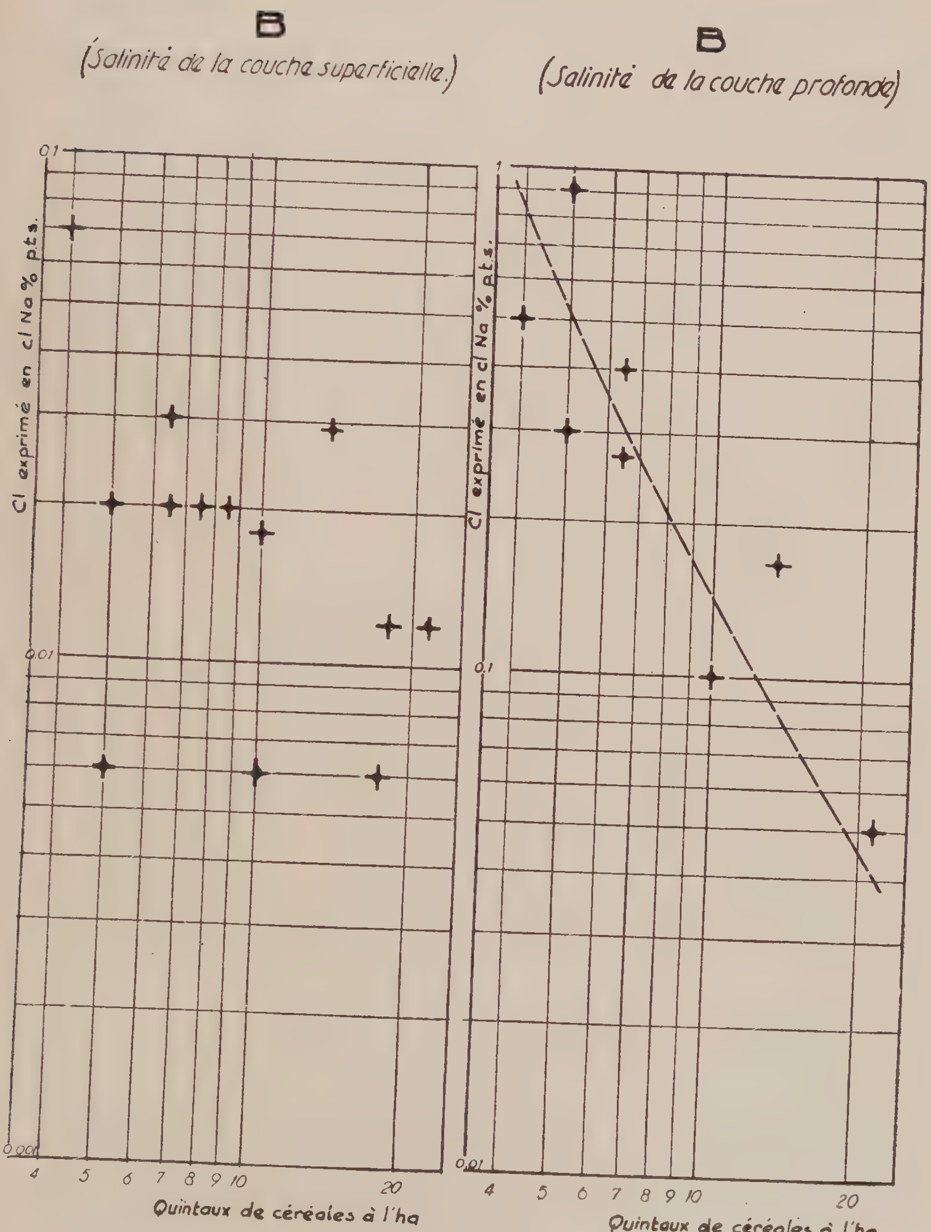
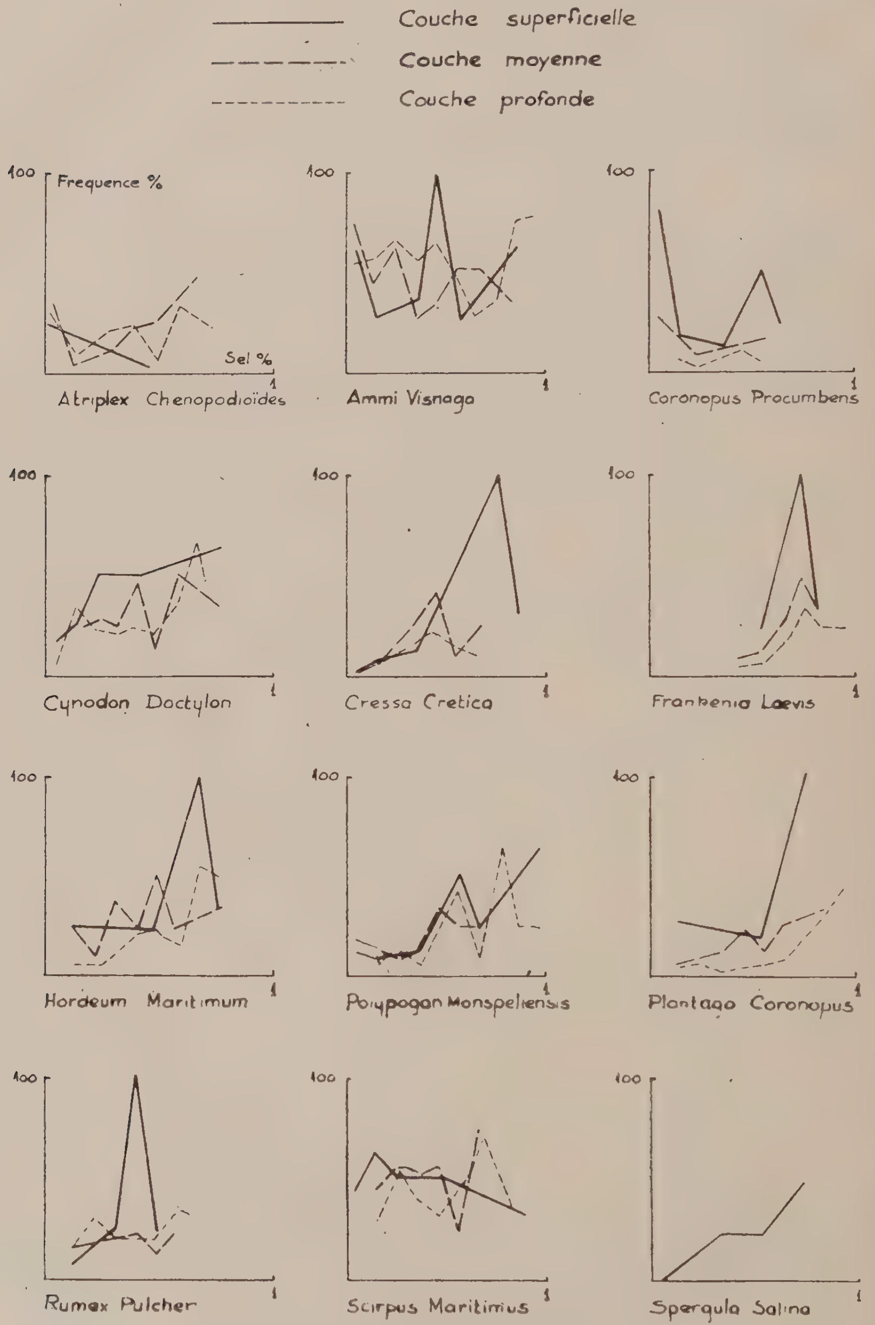


FIGURE 2

FREQUENCE DE PRESENTATION DE CERTAINES ESPECES EN FONCTION DE LA SALINITE DES SOLS



Atriplex Chenopodioïdes · Ammi Visnaga · Coronopus Procumbens

Cynodon Dactylon · Cressa Cretica · Frankenia Laevis

Hordeum Maritimum · Polypogon Monspeliensis · Plantago Coronopus

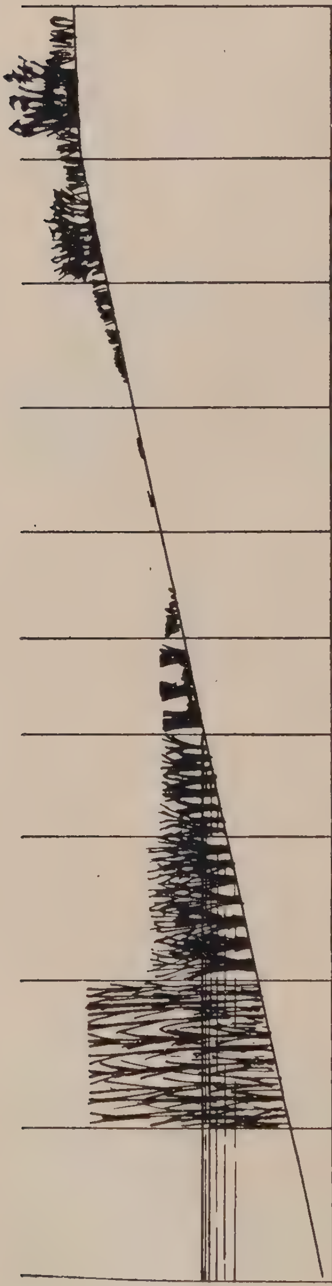
Rumex Pulcher · Scirpus Maritimus · Spergula Salina

TYPE DE SOL SALE	NOM DU GROUPEMENT	ESPECES PRESENTES	OBSERVATIONS
Bassin Argileux et salé % Argile 60 % Sel 0,2 à 0,6 Sol de "TIRS"	Groupement des zones de prairie salée	Polypogon monspeliensis Cressa cretica Hordeum maritimum Cynodon dactylon Plantago coronopus Lepturus cylindricus Spergula salina Damasonium stellatum Coronopus procumbens	Avec prédominance de Polypogon monspeliensis Hordeum maritimum Frankenia laevis Cressa cretica
Levée de rivière Surface Argilolimoneuse Profondeur Sablo-limoneuse % de sel 0,1 à 0,3 Sol de "DESS" anciens lits de rivière en travers des merdjas	Groupement des zones de levée de rivière Groupe à Suaeda	Suaeda fruticosa Polypogon monspeliensis Hordeum maritimum Cynodon dactylon Plantago coronopus Frankenia laevis Spergula salina Cressa cretica Lepturus cylindricus Gaudinia fragilis Gastridum lendigerum	Association en régression du fait du dessalement de la mise en culture et de la surpaturation
Zones à la limite du passage des eaux	Groupe à Juncus acutus	Juncus acutus Agropyrum sp. Festuca elatior Cynodon dactylon Hordeum maritimum Galactites tomentosum	
Limite d'une zone de Bassin et d'une levée de rivière Sol argileux en surface léger en profondeur % de sel 0,1 à 0,6 Tirs sur dess	Groupements des "plages" de Merdja.	Hordeum maritimum Cynodon dactylon Plantago coronopus Frankenia laevis Spergula salina Lolium sp.	

FIGURE 4

INFLUENCE DE LA SALINITE ET DES CONDITIONS HYDRIQUES SUR LA VEGETATION DES LIMITES DE MERDJA

MERDJA BOKKA



Sol	% de sels totaux	Humidité	Espèces caractéristiques
Argileux	0 05	sec	Asphodelus Microcarpus Cortina Sp. Agrostis Sp. Gastridium Lendigerum Gaudinia Fragilis Ammi Visnaga Rumex Pulcher
		sec	Juncus Acutus Hordeum Maritimum Cynodon Dactylon Plantago Coronopus Frankenia Laevis Spergula Salina
Argilo Sableux	0 4	sec	Frankenia Laevis Spergula Salina Plantago Coronopus
	2 à 10	sec	Quelques touffes isolees de Cressa Cretica Frankenia Laevis
		un peu humide	Limonium Ferulaceum Spergula Salina Cressa Cretica
		humide	Juncus Maritimus Spergula Salina
		boueux	Scirpus Maritimus Alisma Plantago Polypogon Monspeliensis
		Faiblement submergé	Typha Latifolia Sparganium Ramosum
	0.2	Faiblement submergé	Phragmites Sp.

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